Synthesis of a series of new racemic [2,3-bis(acyloxy)propyl]phosphonocholines

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Dedicated to Prof. Paweł Kafarski in honor of his scientific achievements within his career

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Abstract

An efficient synthesis of [2,3-bis(acyloxy)propyl]phosphonocholines is described. The reaction pathway for the synthesis involves the Michaelis-Arbuzov reaction of allyl bromide with triethyl phosphite and epoxidation of the resulting diethyl (prop-2-en-1-yl)phosphonate (**1**) with *m*-CPBA to afford the phosphonated diol (**3**). The acylation of diol (**3**) with carboxylic acids gave a series of diacyloxypropylphosphonates (**4**). The introduction of choline to a pyridinium salt of [2,3-bis(acyloxy)propyl]phosphonic acid (**5**) was the most intensively studied reaction. The total yield of a six-step sequence synthesis of a series of phosphonolipids was in the range of 30-40%.

Keywords: Synthesis, phosphonolipids, Michaelis-Arbuzov rearrangement, choline, esterification of phosphonic acid

Introduction

Phosphonolipids constitute important class of organophosphorous compounds and a new class of cationic lipids.[1] The first naturally occurring phosphonolipids were isolated from the single-celled microbes of *Tetrahynema pyriformisare*.[2] To date, phosphonolipids have been discovered in many species of marine animals, various bacteria, certain mammals and other sources.[3,4] These compounds have been extensively studied.[5-7] Generally, phosphonolipids are considered to be components, which protect and stabilize cell-membrane, inhibit phospholipase and facilitate movement of DNA in cells.[8] Additionally, many of these compounds have attracted attention for their biological effects, such as: anticancer, antiviral or enzyme inhibitor properties.[9]

Phosphonolipids, as amphiphilic molecules, can be components of membranes and cell walls, and thus they can be applied in food, cosmetic and pharmaceutical products or in gene transfer therapy.[10-12] There is a distinct possibility to employ phosphonolipids as lipidic prodrugs.[13]

The first syntheses were focused on phosphonolipids with C-P bond on the polar side[14,15] and still, this kind of synthesis of these compounds is the most intensively studied. In the last few years researchers have demonstrated a synthetic route to lysophosphatidic acids (LPA) with C-P bond on the glycerol side.[16,17] LPA reveal a wide variety of responses to cells and tissues. They were used as a factor that regulates cancer cell proliferation, invasion, angiogenesis, metastasis and also biochemical resistance to chemo- and radiotherapy induced apoptosis *via* interactions with G-protein coupled receptors.[18]

One of the factors which determines the physical and biological properties of a lipid is the nature of fatty acids. Therefore, it is important to obtain lipids containing specific fatty acids for scientific purposes and possibly for practical applications.[19-22]

Here, an efficient synthesis of a series of seven racemic [2,3-bis(acyloxy)propyl] phosphonocholines with different fatty acids is presented. The obtained compounds could be considered as phosphono analogs of corresponding diacylophospholipids. In future, these compounds will be tested as substrates in enzymatic hydrolysis and transesterification reactions. Enzymatic reactions carried out on a series of phospho- and phosphonolipids with different aliphatic carbon chains as the hydrophobic part should provide some information concerning the influence of the C-P bond on the activity of phospholipases A_1, A_2 and D. The enzymatic hydrolysis of such molecules is very important in the future of applications of this type of compounds as prodrugs.

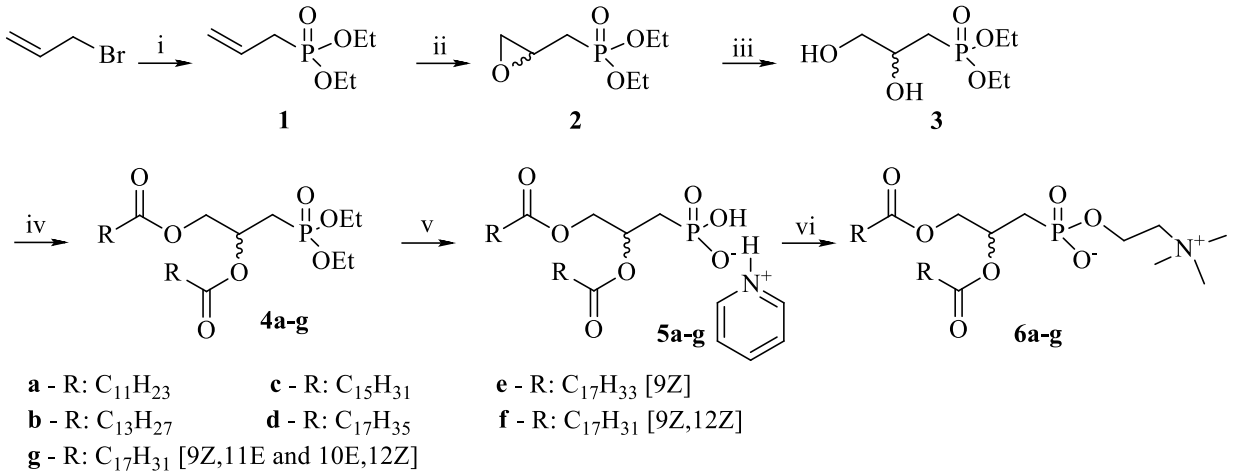
The phosphonate with CLA was synthesized because of many biological activities of CLA itself. It exhibited a considerable anti-proliferative effect on cancer cells in *in vitro* study and anti-tumor activity in *in vivo* tests.[23]

Results and Discussion

The synthesis of [2,3-bis(acylo)propyl]phosphonocholines (**6a-g**) is shown in Scheme 1.

The Michaelis-Arbuzov rearrangement was the first step of the synthesis. The reaction of allyl bromide with triethyl phosphite afforded diethyl (prop-2-en-1-yl)phosphonate (**1**) in a very high, practically quantitative, yield. The reaction was facilitated by an excess of allyl bromide (10%), which can be easily removed by distillation. The first attempts with of these reactions were carried out with the addition of iodide salt (NaI, KI). It is known that alkyl iodides are more reactive than bromides and these salts were very often used to accelerate the reaction.[24] In our experiments with application of NaI or KI the yields were very high, but the product of the reaction had an undesirable dark color which caused the necessity of additional purification on a silica gel column. Therefore, we decided to perform this reaction without iodide salt. The yields of the trials were also very high and let us avoid purification of the product. The structure of

diethyl allylphosphonate (**1**) was confirmed by NMR data. The presence of the allyl group confirms the signals of the olefin protons: multiplet of H-2 at 5.78 ppm and multiplet of CH_2-3 in the range of 5.23-5.16 ppm. Methylene protons of CH_2-1 gave in the spectrum doublet (J = 21.9 Hz) of doublets (J = 7.4 Hz) of triplets (J = 1.2 Hz).



Scheme 1. General synthesis of new series of phosphonolipids. Reagents and conditions: (i) $P(OEt)_3$, reflux, 5 h; (ii) *m*-CPBA, CH_2Cl_2, 48 h; (iii) aq CH_3COOH; (iv) $R_{a\text{-}g}COOH$, DCC, DMAP, CH_2Cl_2, rt, 48 h; (v) a) TMSBr, 95% MeOH, 4 h; b) H^+ - Dowex resin, Dowex resin - pyridinium form; (vi) choline tosylate, DCC, pyridine, rt, 48 h.

In the next step, the epoxidation of the diethyl (prop-2-en-1-yl)phosphonate (**1**), with 1.5 equiv of 3-chloroperbenzoic acid (*m*-CPBA) was carried out. The epoxyphosphonate (**2**) was obtained with 92% yield. The multiplets of H-2 (3.18 ppm) and CH_2-3 (2.82 and 2.58 ppm) confirmed the presence of an oxirane ring in the product.

The aqueous or THF (95%) solutions of HCl, H_2SO_4, $HClO_4$ and acetic acid were tried as reagents for opening the oxirane ring in diethyl (2,3-epoxypropyl)phosphonate (**2**). The best results were obtained for acetic acid solution (pH<2) in water. The diethyl (2,3-duhydroxypropyl)phosphonate (**3**) was obtained in a 79% yield. Other experiments gave lower yields. The structure of diethyl (2,3-dihydroxypropyl)phosphonate (**3**) was confirmed by its spectral data (^{1}H and ^{13}C NMR, MS). The multiplet of H-2 proton was observed in the ^{1}H-NMR spectra together with multiplets of $–OCH_2$ protons in the range of 4.23 – 4.01 ppm and the doublets of doublets of CH_2-3 are located at 3.70 and 3.54 ppm. The attempts of obtaining (2,3-dihydroxypropyl)phosphonate (**3**) by direct oxidation of diethyl allylphosphonate (**1**) with potassium permanganate in water as well as acetone solution failed. A non-separable complex mixture of products was obtained.

The acylation of diethyl (2,3-dihydroxypropyl)phosphonate (**3**) with carboxylic acids was carried out in the presence of coupling agent (DCC). The molar excess of carboxylic acid per one mole of diol (**3**) was established in the experiments on the synthesis of diethyl [2,3-

bis(palmitoyloxy)propyl]phosphonate (**4c**). The trials were carried out with 2.4, 3.0 and 4.0 equiv. of acid pere one mole of phosphonate (**3**). The results showed that the highest yield (92%) of product was obtained in the reaction with 3.0 molar equiv. of acid per one mole of diethyl (dihydroxypropyl)phosphonate (**3**).

Other conditions of the reaction of phosphonates (**3-6**) with fatty acids were also worked out in experiments with palmitic acid (PA) (**4c-6c**). The symmetrical diethyl [2,3-bis(acyloxy)propyl]phosphonates (**4**) were prepared by direct acylation of hydroxyl groups of diol (**3**) with the 3.0 molar excess (1.5 per one hydroxyl group) of appropriate carboxylic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in dry CH_2Cl_2.

The purity of the phosphonates (**4a-g**) was confirmed by HPLC (Figure 1, Chromatogram A). In the course of these analyses the influence of the carbon chain length of carboxylic acid on the retention time (R_t) of phosphonates (**4a-g**) was observed. Increase in the length of the carbon chain of fatty acid leads to higher R_t (HPLC: ODS2 Sperisorb column). In contrast to double bond in the rest of oleic acid (**4e**), linoleic acid (**4f**) and conjugated linoleic acid (**4g**) decrease their R_t in comparison to the R_t of diethyl [2,3-bis(stearoyloxy)propyl]phosphonate (**4d**) (Figure 1, Chromatogram B).

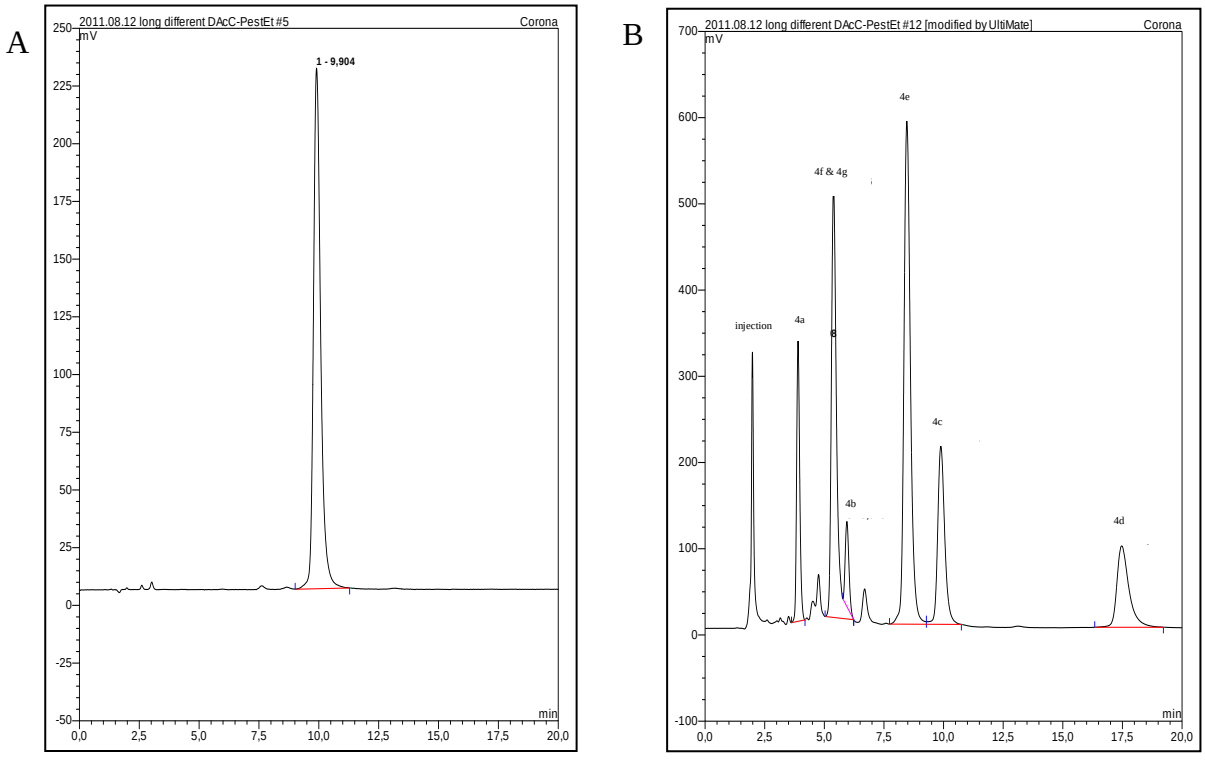


Figure 1. Reversed phase of [2,3-bis(palmitoyl)propyl]phosphonate (**4c**) on ODS2 Sperisorb column (A); chromatograms of **4a-g** (B). HPLC analysis with detection via ESA Corona™ CAD.

The structures of diacyl phosphonates (**4a-g**) were confirmed by their spectral data (^{1}H and ^{13}C NMR, ^{31}P NMR). Comparing the ^{1}H NMR spectra of both substrate and products it is noticed that the signals from the proton H-2 were shifted downfield from 4.23 – 4.10 ppm in the spectrum of diol (**3**) to above 5 ppm for [2,3-bis(acyloxy)propyl]phosphonates (**4a-g**). Similarly, the signals belonging to CH_2-3 protons were shifted from 3.70 and 3.54 ppm to above 4 ppm. Structures of the fatty acids residue were also confirmed by spectral data. The signals of the olefin protons were in the same region of the spectrum as signals of H-2. ^{1}H NMR and ^{13}C NMR spectra of (**4g**) were analyzed according to earlier studies.[25]

Esterification of (2,3-epoxypropyl)phosphonate (**2**) with palmitic acid anhydride was also tested. The reaction was carried out according to the procedure of Ali and Bittman.[26] The best yield (57.6%) of (**4c**) was obtained. So we decided to apply a two-step synthesis of phosphonates (**4a-g**) *via* diol (**3**).

The transformation of diethyl [2,3-bis(acyloxy)propyl]phosphonates (**4**) to phosphonic acid required a careful choice of reagent. Bromotrimethylsilane (TMSBr) is commonly used for this purpose.[27,28] After several experiments carried out in different solvents (CH_3CN or CH_2Cl_2), at several temperatures (20, 35 ºC and under reflux) and with various molar excesses of TMSBr we decided to carry out this reaction in CH_2Cl_2 with a 10 molar excess of TMSBr at room temperature. The [2,3-bis(acyloxy)propyl]phosphonic acids were very unstable so they were transformed to more stable and reactive form of monopyridinium salts (**5a-g**)[24,29,30]

The ^{1}H and ^{13}C NMR spectral data of the pyridinium salts (**5a-g**) confirmed their structures. The signals of aromatic protons are present in the ^{1}H NMR spectrum at approximately 8.6, 8.0, 7.5 ppm and the signals of pyridine carbon atoms at 148.5, 137.7 and 124.5 ppm.

Table 1. The yields (according to HPLC) of syntheses of [2,3-bis(acyloxy)propyl]phosphonocholine (6c) from different forms of phosphonic acid and in various solvents

Entry	Substrates	Solvent	Yields [%]
1	A	pyridine	1
2	A	CH_2Cl_2	1
3	B	pyridine	51
4	B	CH_2Cl_2	37
6	B	CCl_3CN	54
7	B	TEA	2
8	B	toluene	43

Reaction conditions: 1eq of phosphonic acid (A) or monopyridinium salt of phosphonic acid (**5c**) (B), choline tosylate (2 molar eq), DCC (3 molar eq), 48 h, room temp., N_2, solvent (TEA - trietylamine)

The introduction of choline moiety into [2,3-bis(acyloxy)propyl]phosphonic acid was achieved in the reaction of pyridinium salt of phosphonic acid (**5a-g**) with choline tosylate (Scheme 1). The reaction of phosphonic acid with an active molecule, such as choline, is usually the crucial step in the synthesis of modified phospho- and phosphonolipids, including prodrugs.[13,31] The first experiments on this reaction were carried out for [2,3-bis(palmitoyloxy)propyl]phosphonic acid and its pyridinium form (**5c)** in the presence of DCC (3 molar excess) as coupling agent in dry pyridine or in dry CH_2Cl_2. Results presented in Table 1 showed that the protonic form of phosphonic acid was not a good substrate for synthesis of **6c**. The yield of the reaction was below 2%. The pyridinium salt form (**5c**) was a much more reactive substrate.[29] The yields of the reaction were in the range of 37-51% (Table 1). So in the next experiments pyridinium salt of phosphonic acid (**5c**) was used.

The results of the esterification carried out in various solvents (CH_2Cl_2, pyridine, CCl_3CN, toluene, trietylamine) led to the conclusion that the best results were obtained for pyridine and CCl_3CN. We decided to use pyridine as a solvent for the next reactions, because the pyridine dissolves the substrates and the products more effectively. The molar excess of choline tosylate was also established in the experiments on synthesis of (**6c**). The common condition used for esterification of phosphatidic acid with choline needs the use of 10 molar excess of choline.[26] In our method the 2 molar excess of choline tosylate was sufficient. It is a result of the application of the more reactive form (pyridinium salt) of phosphonic acid. Three coupling agents were tested in the reaction: *N*,*N*'-dicyclohexylcarbodiimide (DCC), p-toluenesulfonyl chloride (TsCl) and mesitylene-sulfonyl chloride (MeCl). The DCC was the most effective with a yield of 50,8%. Conditions elaborated for the reaction of the introduction of choline to the phosphonic acid molecule let us obtain the phosphonates (**6a-g**) in good yields (60 – 72%). Structures of [2,3-bis(acyloxy)propyl]phosphonocholine (**6a-g**) were confirmed by their NMR spectral data. Signals from protons of the choline group were present in the ^{1}H NMR spectra as a multiplets cumulated between 4.5 – 3 ppm. A singlet of $–N(CH_3)_3$ group protons was observed at about 3.17 ppm.

Conclusions

An efficient method of synthesis of phosphonolipids with choline as a polar head was elaborated. The introduction of the choline to phosphonic acid in known methods requires a high excess of choline, even 10 molar equiv.[26] Lower excess of choline moiety could be applied for the synthesis with a more reactive form – pyridinium salt of phosphonic acid (**5**). Our method allows to use only 2 molar equiv. of choline tosylate. The presented method could be applied for synthesis of different phosphonolipids as potential prodrugs.

We hope that studies on enzymatic hydrolysis of a series of phosphonolipids with different aliphatic carbon chains as the hydrophobic part can afford interesting information on influence of fatty acid carbon chain length on the efficiency of enzymatic hydrolysis of such compounds.

Comparable studies on hydrolysis of phospholipids and their phosphono analogues by phospholipase A_1, A_2, D could afford valuable information on the influence of C-P bond on these enzyme activity.

Experimental Section

General: All reagents with *m*-chloroperbenzoic acid (77%) were purchased from Sigma-Aldrich or Fluka (Poland). All solvents and reagents were of analytical grade. TLC analyses were carried out on silica gel-coated aluminium plates (DC–Alufolien Kieselgel 60 F254, Merck) with various solvent systems as eluent. Compounds were detected by spraying the plates with 0.05% primuline solution in a mixture of acetone:water (8:2 v/v) or 1% $Ce(SO_4)_2$, 2% $H_3[P(Mo_3O_{10})_4]$ in 10% H_2SO_4. Column chromatography was performed on silica gel (Kieselgel 60, 230–400 mesh ASTM, Merck) with various solvent systems.

ESI-MS were measured on a Bruker micrOTOF-Q and GC-MS analyses were measured on a mass spectrometer (MS), equipped with an ion-trap analyzer, set at 1508 for all analyses with an electron multiplier voltage of 1350 V. Scanning (1 scan s^{-1}) was performed in the range of 39–400 *m/z* and using electron impact ionization at 70eV.

NMR spectra were measured on a Bruker Avance II 600 MHz. Chemical shifts (^1H and ^{13}C) (δ) are given in ppm with TMS as the internal standard, in ^{31}P NMR chemical shifts were referenced to 85% H_3PO_4 as an external standard. Coupling constant (*J*) values are in Hz. Assignments of signals to corresponding protons and carbons were made on the basis of homo- and heteronuclear correlation (^1H-^1H COSY, ^1H-^{13}C HMQC).

Gas chromatography analysis was carried out on a Varian CP-3380 instrument (FID, carrier gas H_2) using TR-5 (30 m x 0.32 mm x 1.0 μm) with the following temp. program: 110 –210°C at 30°C/min, then with rate 30°C/min to 300°C and hold for 1 min. The total run time was 9.6 min.

The HPLC analyses were performed on Ultimate 3000 from DIONEX with autosampler and ESA Corona™ Charged Aerosol Detector from ESA Biosciences, detector operated with nitrogen as a nebulizing gas and at a range of 100 pA. The Betasil DIOL 5 μm column (Thermo, 150 x 2,1 mm) and ODS2 Sperisorb (Waters, 150 mm x 4,6 mm x 3 μm) were used. The gradient for Betasil DIOL 5 μm column had a constant flow rate of 0.6 mL/min, with solvent A = water (1% formic acid, 0.1% triethylamine), B = hexane, C = isopropanol. Gradient timetable: at 0 min, 8:40:52 (%A:%B:%C v/v/v); at 3 min 8:40:52; at 6 min, 10:40:50; at 11 min, 10:40:50; at 16 min, 13:37:50; at 25 min 13:37:50, at 25.5 min, 8:40:52. The gradient for ODS2 Sperisorb (Waters, 150 mm x 4,6 mm x 3 μm) column had a constant flow rate of 1.0 mL/min, with isocratic solvent A = water (1% formic acid), B = acetonitryle, C = isopropanol, 5:45:50 (%A:%B:%C v/v/v).

Diethyl (prop-2-en-1-yl)phosphonate (1). To a round-bottomed flask equipped with a reflux condenser and under a nitrogen atmosphere freshly distilled triethyl phosphite (5.16 mL, 30 mmol) and allyl bromide (5.52 mL, 33 mmol) was added. The mixture was heated to 71 °C, 3 h. Then the excess of allyl *bromide* was distilled off by heating the crude mixture in the range of

about 120 °C for 2 h. Ester (1) as a colourless oil (5.25 g, 98%) was obtained. The purity of the product was determined by TLC and GC. Their physical and spectral data are as follows:
TLC R_f = 0.41 ($CHCl_3$:MeOH 45:1 v/v); GC R_t = 4.02 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.30 (t, *J* = 7.1 Hz, 6H, 2x -OCH_2CH_3), 2.60 (ddt, *J* = 21.9, 7.4, 1.2 Hz, 2H, CH_2-P), 4.14 – 4.04 (m, 4H, 2x -OCH_2CH_3), 5.23-5.16 (m, 2H, CH=CH_2), 5.78 (m, 1H, CH=CH_2); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 16.44 and 16.40 (-OCH_2CH_3), 32.25 (d, *J* = 138.3 Hz, CH_2-P), 61.95 and 61.90 (-OCH_2CH_3), 119.95 (d, *J* = 14.4 Hz, =CH_2), 127.56 (d, *J* = 11.2 Hz, =CH-); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 27.08; EIMS (%): 41.2 (17.5), 65.2 (17.5), 81.2 (33.7), 91.2 (22.1), 97.1 (31.2), 109.1 (99.9), 123.2 (27.4), 134.0 (38.5), 151,1 (31.7), 178.8 [M+] (89.9).

Synthesis of diethyl (2,3-epoxypropyl)phosphonate (2). A solution of *m*-chloroperbenzoic acid (7.63 g, 2.65 mmol) in CH_2Cl_2 (175 mL) was added dropwise to an ice-cooled and stirred solution of the **(1)** (5.29 g, 29 mmol) in CH_2Cl_2 (200 mL) during 3 h. After this time the ice bath was removed and the mixture was stirred for 48 h at room temperature. When the reaction was completed (GC, TLC) the excess of *m*-chloroperbenzoic acid was reduced with 10% $Na_2S_2O_3$ solution, the organic layer was washed with 10% Na_2CO_3, brine and dried over anhydrous $MgSO_4$. The crude product was purified on a silica gel column (eluent: $CHCl_3$:MeOH 35:1 v/v) to give appropriate, colourless oil, of diethyl (2,3-epoxypropyl)phosphonate (**2**) (5.26 g, 92%). Purity (above 98%) was confirmed by GC. Their physical and spectral data are as follows:
TLC R_f = 0.33 ($CHCl_3$:MeOH 35:1 v/v); GC R_t = 5.30 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.34 (two t, *J* = 7.1 Hz, 6H, 2x -OCH_2CH_3), 1.85 (ddd, *J* = 19.9, 15.2, 6.5 Hz, 1H, one of CH_2-P), 2.21 (ddd, *J* = 18.3, 15.2, 5.6 Hz, 1H, one of CH_2-1), 2.58 (dd, *J* = 4.9, 2.5 Hz, 1H, one of CH_2-3), 2.82 (m, 1H, one of CH_2-3), 4.23 – 3.98 (m, 4H, 2x -OCH_2CH_3), 3.18 (m, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 16.47 and 16.43 (-OCH_2CH_3), 30.69 (d, *J* = 138.3 Hz, CH_2-P), 46.85 (d, *J* = 2.2 Hz, CH-2), 47.33 (d, *J* = 7.3 Hz, CH_2-3), 61.98 and 61.93 (-OCH_2CH_3); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 26.16; EIMS (%): 47.2 (24.9), 57.1 (19.7), 65.2 (52.7), 81.1 (37.0), 97.1 (25.5), 111.1 (84.4), 121.2 (67.4), 138.9 (99.9), 148.9 (11.5), 164.9 (34.4), 194.8 [M+] (78.9).

Synthesis of diethyl (2,3-dihydroxypropyl)phosphonate (3). Solution of epoxide (**2**) (5,26 g, 27 mmol) in 20 mL of water was acidified by acetic acid to pH<2. The mixture was stirred at room temperature. When the reaction was completed (GC) the solution was evaporated *in vacuo* and the residue was co-evaporated several times from $CHCl_3$:MeOH (2:1 v/v). Crude product was purified by silica gel column chromatography (eluent: $CHCl_3$:MeOH 15:1 v/v). Colourless oil of diethyl (2,3-dihydroxypropyl)phosphonate (**3**) (4.58 g, 79%) was obtained. Its physical and spectral data are given below:
TLC R_f = 0.18 ($CHCl_3$:MeOH 15:1 v/v); GC R_t = 6.29 min; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34 (two t, *J* = 7.1 Hz, 6H, 2x -OCH_2CH_3), 1.94 (dd, *J* = 19.0, 15.7, 6.3 Hz, 1H, one of CH_2-1), 2.05 (dd, *J* = 18.1, 15.7, 5.5 Hz, 1H, one of CH_2-1), 3.54 (dd, *J* = 11.4, 5.6 Hz, 1H, one of CH_2-3), 3.70 (dd, *J* = 11.4, 3.6 Hz, 1H, one of CH_2-3), 4.23 – 4.01 (m, 5H, H-2 and 2x -OCH_2CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 16.42 and 16.39 (-OCH_2CH_3), 30.27 (d, *J* = 140.5 Hz, CH_2-P), 62.20 and 62.16 (-OCH_2CH_3), 67.06 (d, *J* = 16.4 Hz, CH_2-3), 66.77 (C-2); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 30.26; EIMS (%): 47.2 (35.9), 57.1 (15.1), 65.2 (74.6), 80.1 (37.2), 97.1 (32.2), 111.1

(73.1), 121.2 (47.2), 125.1 (83.0), 139.1 (99.9), 149.1 (32.4), 167.0 (54.9), 181.0 (41.6), 213.0 [M+] (14.0).

Synthesis of diethyl (2,3-bis(acyloxy)propyl]phosphonates (4a-g). A solution of 1,3-dicyclohexylcarbodiimide (613 mg, 2.97 mmol) in dry CH_2Cl_2 (5 mL) was added to a solution of diethyl phosphonate (**3**) (200mg, 0.94 mmol), appropriate fatty acid (**a-g)** (2.83 mmol), and (dimethylamino)pyridine (35 mg, 0.28 mmol) in dry CH_2Cl_2 (12 mL) and was stirred under nitrogen, for 48 h at room temperature. Next the reaction mixture was filtered through Celite and evaporated. Then the crude product was dissolved in $CHCl_3$:MeOH:H_2O (5:4:1 v/v/v) and DOWEX 50W X8 (H^+-form) was added to remove 4-(dimethylamino)pyridine. The solution was stirred for 30 min, ion exchange resin was filtered out and the solvent was evaporated *in vacuo*. The product was purified on silica gel (eluent: hexane:ethyl acetate 2:1 v/v). The spectral data of obtained compounds 4a-g are given below:

Diethyl [2,3-bis(lauroyloxy)propyl]phosphonate (4a). White solid; m_p=7^{0}C, yield 90%, 488 mg; TLC R_f = 0,36 (hexane:ethyl acetate 2:1 v/v), HPLC: ODS2 Sperisorb, R_t=3.90 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.88 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_{10}CH_3$), 1.31-1.23 (m, 32H, 2x $(CH_2)_8CH_3$), 1.33 (two t, *J* = 7.1 Hz, 6H, 2x OCH_2CH_3), 1.63-1.58 (m, 4H, 2x CH_2CH_2COO), 2.14 (dd, *J* = 19.0, 6.8 Hz, 2H, CH_2-1), 2.31 (m, 4 H, 2x CH_2COO), 4.17 – 4.06 (m, 5H, 2x -OCH_2CH_3 and one of CH_2-3), 4.36 (dd, *J* = 12.0, 3.3 Hz, 1H, one of CH_2-3), 5.33 (m, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.12 (two $(CH_2)_{10}CH_3$); 16.43 and 16.39 (OCH_2**C**H_3), 22.69 (one of (**C**$H_2)_8$), 24.90 and 24.82 (**C**H_2CH_2COO), 28.35 (d, *J* = 144.9 Hz, C-1), 31.92 – 29.13 (signals of (**C**$H_2)_8$), 34.29 and 34.12 (CH_2COO), 62.06 and 62.00 ($P(OCH_2CH_3)_2$), 66.63 – 64.56 (C-2 and C-3), 173.29 and 172.74 (C(O)O). ^{31}P NMR (243 MHz, $CDCl_3$) δ: 25.60; HR-MS *m/z*: 577.2237 $[M+H]^+$.

Diethyl [2,3-bis(myristoyloxy)propyl]phosphonate (4b). White solid; m_p=22^{0}C, yield 92%, 547 mg; TLC R_f = 0,36 (hexane:ethyl acetate 2:1 v/v), HPLC: ODS2 Sperisorb, R_t=5.95 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.88 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_{12}CH_3$), 1.31-1.23 (m, 40H, 2x $(CH_2)_{10}CH_3$), 1.33 (two t, *J* = 7.1 Hz, 6H, 2x OCH_2CH_3), 1.63-1.58 (m, 4H, 2x CH_2CH_2COO), 2.14 (dd, *J* = 19.0, 6.8 Hz, 2H, CH_2-1), 2.31 (m, 4 H, 2x CH_2COO), 4.17 – 4.06 (m, 5H, 2x -OCH_2CH_3 and one of CH_2-3), 4.36 (dd, *J* = 12.0, 3.3 Hz, 1H, one of CH_2-3), 5.32 (m, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.12 (two $(CH_2)_{12}CH_3$), 16.43 and 16.39 (OCH_2CH_3), 22.69 (one of $(CH_2)_{10}$), 24.90 and 24.82 (CH_2CH_2COO), 28.35 (d, *J* = 144.5 Hz, C-1), 31.92 – 29.13 (signals of $(CH_2)_{10}$), 34.29 and 34.12 (CH_2COO), 62.06 and 62.00 ($P(OCH_2CH_3)_2$), 66.63 – 64.56 (C-2 and C-3), 173.29 and 172.74 (C(O)O). ^{31}P NMR (243 MHz, $CDCl_3$) δ: 25.60; HR-MS *m/z*: 633.4889 $[M+H]^+$.

Diethyl [2,3-bis(palmitoyloxy)propyl]phosphonate (4c). White solid; m_p=44^{0}C, yield 92%, 596 mg; TLC R_f = 0,35 (hexane:ethyl acetate 2:1 v/v), HPLC: ODS2 Sperisorb, R_t=9.90 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.88 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_{14}CH_3$), 1.31-1.23 (m, 48H, 2x $(CH_2)_{12}CH_3$), 1.33 (two t, *J* = 7.1 Hz, 6H, 2x OCH_2CH_3), 1.63-1.58 (m, 4H, 2x CH_2CH_2COO), 2.13 (dd, *J* = 19.0, 6.8 Hz, 2H, CH_2-1), 2.31 and 2.29 (two t, *J* = 6.9, 4H, 2x CH_2COO), 4.17 – 4.08 (m, 5H, 2x -OCH_2CH_3 and one of CH_2-3), 4.36 (dd, *J* = 12.0, 3.3 Hz, 1H, one of CH_2-3),

5.35 (m, 1H, H-2). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.13 (two $(CH_2)_{14}CH_3$), 16.43 and 16.40 (OCH_2CH_3), 22.70 (one of $(CH_2)_{12}$), 24.91 and 24.82 (CH_2CH_2COO), 28.37 (d, *J* = 141.0 Hz, C-1), 31.94 – 29.14 (signals of $(CH_2)_{12}$), 34.29 and 34.12 (CH_2COO), 62.02 and 69.96 ($P(OCH_2CH_3)_2$), 66.64 – 64.61 (C-2 and C-3), 173.28 and 172.73 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 25.55; HR-MS *m/z*: 689.5471 $[M+H]^+$.

Diethyl [2,3-bis(stearoyloxy)propyl]phosphonate (4d). White solid; m_p=58^{0}C, yield 87%, 609 mg; TLC R_f = 0,35 (hexane:ethyl acetate 2:1 v/v), HPLC: ODS2 Sperisorb, R_t=17.5 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.88 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_{16}CH_3$), 1.31-1.23 (m, 56 H, 2x $(CH_2)_{14}CH_3$), 1.33 (two t, *J* = 7.1 Hz, 6H, 2x OCH_2CH_3), 1.63-1.58 (m, 4H, 2x CH_2CH_2COO), 2.13 (dd, *J* = 19.0, 6.8 Hz, 2H, CH_2-1), 2.31 and 2.29 (two t, *J* = 6.9, 4H, 2x CH_2COO), 4.17 – 4.08 (m, 5H, 2x -OCH_2CH_3 and one of CH_2-3), 4.36 (dd, *J* = 12.0, 3.3 Hz, 1H, one of CH_2-3), 5.34 (m, 1H, H-2);^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.13 (two $(CH_2)_{16}CH_3$), 16.43 and 16.40 (OCH_2CH_3), 22.70 (one of $(CH_2)_{14}$), 24.91 and 24.82 (CH_2CH_2COO), 28.37 (d, *J* = 140.2 Hz, C-1), 31.94 – 29.14 (signals of $(CH_2)_{14}$), 34.29 and 34.12 (CH_2COO), 62.02 and 69.96 ($P(OCH_2CH_3)_2$), 66.64 – 64.61 (C-2 and C-3), 173.28 and 172.73 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 25.55; HR-MS *m/z*: 745.6214 $[M+H]^+$.

Diethyl [2,3-bis(oleoyloxy)propyl]phosphonate (4e). Colourless oil; yield 84%, 585 mg; TLC R_f = 0,35 (hexane:ethyl acetate 2:1 v/v), HPLC: ODS2 Sperisorb, R_t=8.48 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.88 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_7CH_3$), 1.31 – 1.24 (m, 40H, 20x CH_2), 1.33 (two t, *J* = 7.1 Hz, 6H, 2x OCH_2CH_3), 1.64-1.58 (m, 4H, 2x CH_2CH_2COO), 2.00 (m, 8H, 4x $=CHCH_2$), 2.13 (dd, *J* = 19.0, 6.8 Hz, 2H, CH_2-1), 2.31 and 2.30 (two t, *J* = 6.9Hz, 4H, 2x CH_2COO), 4.19 – 4.02 (m, 5H, 2x -OCH_2CH_3 and one of CH_2-3), 4.36 (dd, *J* = 12.0, 3.3 Hz, 1H, one of CH_2-3), 5.40 – 5.26 (m, 5H, H-2 and 2x -CH=CH-); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.12 (two $(CH_2)_7CH_3$), 16.44 and 16.39 (OCH_2CH_3), 22.69 (one of $(CH_2)_{13}$), 35.98 – 24.80 (signals of $(CH_2)_{13}$ and C-1), 34.26 and 34.09 (CH_2COO), 62.00 and 61.96 ($P(OCH_2CH_3)_2$), 66.66 – 64.56 (C-2 and C-3), 130.04 and 129.72 (CH=CH), 173.24 and 172.70 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 25.54; HR-MS *m/z*: 741.6083 $[M+H]^+$.

Diethyl [2,3-bis(linoleoyloxy)propyl]phosphonate (4f). Yellow oil; yield 86%, 596 mg; TLC R_f = 0,34 (hexane:ethyl acetate 2:1 v/v), HPLC: ODS2 Sperisorb, R_t=5.37 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.89 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_4CH_3$), 1.40 – 1.21 (m, 30H, 2x OCH_2CH_3 and 12x CH_2), 1.65 – 1.55 (m, 8H, 2x CH_2CH_2COO and 2x $=CHCH_2CH_2$), 2.04 (m, 8 H, 4x $=CHCH_2$), 2.13 (dd, *J* = 19.0, 6.8 Hz, 2H, CH_2-1), 2.34 – 2.25 (m, 4H, 2x CH_2COO), 2.77 (two t, *J* = 6.8 Hz, 4H, -2x $=CHCH_2CH=$), 4.17 – 4.07 (m, 5H, 2x -OCH_2CH_3 and one of CH_2-3), 4.36 (dd, *J* = 12.0, 3.3 Hz, 1H, one of CH_2-3), 5.42 – 5.28 (m, 9H, H-2 and 4x -CH=CH-); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.08 (two $(CH_2)_4CH_3$), 16.44 and 16.40 (OCH_2CH_3), 22.59 (one of $(CH_2)_{10}$), 25.64 (two $=CHCH_2CH=$), 31.54 – 24,79 (signals of $(CH_2)_{10}$ and C-1), 34.26 and 34.08 (CH_2COO), 62.07 and 62.00 ($P(OCH_2CH_3)_2$), 66.66 – 64.63 (C-2 and C-3), 128.09 and 127.91 (CH=CH), 130.25 and 130.02 (CH=CH), 173.23 and 172.69 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 25.54; HR-MS *m/z*: 737.5466 $[M+H]^+$.

Diethyl [2,3-bis(conjugated linoleoyloxy)propyl]phosphonate (4g). Colourless oil; yield 89%, 617 mg; TLC R_f = 0,34 (hexane:ethyl acetate 2:1 v/v), HPLC: ODS2 Sperisorb, R_t=5.41 min; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.91 – 0.85 (m, 6H, 2x $(CH_2)_4CH_3$), 1.41 – 1.24 (m, 38H, 2x OCH_2CH_3 and 16x CH_2), 1.64 – 1.56 (m, 8H, 2x CH_2CH_2COO and 2x $=CHCH_2CH_2$), 2.18 – 2.06 (m, 10H, CH_2-1 and 4x $=CHCH_2$), 2.31 and 2.30 (two t, *J* = 6.7 Hz, 4H, 2x CH_2COO), 4.18 – 4.06 (m, 5H, 2x $-OCH_2CH_3$ and one of CH_2-3), 4.36 (dd, *J* = 12.0, 3.3 Hz, 1H, one of CH_2-3), 5.37 – 5.24 (m, 3H, H-2, 2x one of =CH; H-9' and H-13"), 5.65 (m, 2H, 2x one of =CH; H-12' and H-10"), 5.94 (m, 2H, 2x one of =CH; H-10' and H-12"), 6.32 – 6.24 (m, 2H, H-11' and H-11"); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.11 and 14.07 (two $(CH_2)_4$**C**H_3), 16.44 and 16.40 (OCH_2**C**H_3), 35.36 – 22.63 (signals of $(CH_2)_{24}$ of 9*c*,11*t*-CLA and 10*t*,12*c*-CLA and C-1), 62.07 - 61.96 ($P(O$**C**$H_2CH_3)_2$), 66.65 – 64.62 (C-2 and C-3), 125.56 (C-11'), 125.68 (C-11"), 128.57 (C-12"), 129.88 (C-9'), 128.71 (C-10'), 130.19 (C-13"), 134.57 (C-10"), 134.83 (C-12'), 173.24 and 172.70 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$) δ: 25.54; HR-MS *m/z*: 737.5463 $[M+H]^+$.
(') – signals from (9*c*, 11*t*)-conjugated linoleic acid (CLA)
(") - signals from (10*t*, 12*c*)-conjugated linoleic acid

Synthesis of monopyridinium salt of [2,3-bis(acyloxy)propyl]phosphono acid (5a-g)
Dried diethyl (2,3-bis(acyloxy)propyl]phosphonates (**4a-g**) (0,50 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and stirred at room temperature under nitrogen. Bromotrimethylsilane (0,66 mL, 5,00 mmol) was added and the mixture was stirred for 4 h. Then, the solvent was removed *in vacuom.* The residue was dissolved in 95% MeOH (12 mL) for 1 h and reconcentrated in vacuo to give a 2,3-bis(acyloxy)propyl]phosphono acid. The phosphonic acid was firstly dissolved in $CHCl_3$:MeOH:H_2O (5:4:1 v/v/v) and applied to a column of DOWEX 50W X8 (H^+ form) resin, evaporated *in vacuo*. Then, it was dissolved in 7 mL of $CHCl_3$:MeOH:pyridine:H_2O (3:3:1:1 v/v/v/v) and filtered through a column DOWEX 50W X8 (pyridinium form) resin. The required product was eluted with the same solvent, the solution was evaporated *in vacuo* and the residue was co-evaporated several times from $CHCl_3$:MeOH (2:1 v/v). The product was evaporated from benzene to give (2,3-bis(acyloxy)propyl]phosphonic acid (**5a-g**) in monopyridinium form (yield >90%). The spectral data of obtained compounds **5a-g** are given below:
Monopyridinium salt of [2,3-bis(lauroyloxy)propyl]phosphonate (5a). White solid; m_p=78^{0}C, yield 90%, 270 mg; TLC R_f = 0.22 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 12,6 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.87 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_{10}CH_3$), 1.31-1.23 (m, 32H, 2x $(CH_2)_8CH_3$), 1.61-1.57 (m, 4H, 2x CH_2CH_2COO), 2.14 (dd, *J* = 18.5, 6.3 Hz, CH_2-1), 2.30 (m, 4 H, 2x CH_2COO), 4.17 – 4.06 (m, 1H, one of CH_2-3), 4.36 (m, 1H, one of CH_2-3), 5.36 (m, 1H, H-2), 7.47 (m, 2H, 3,5-C_5H_5N), 7.89 (t, *J* = 7.5 Hz, 1H, 4-C_5H_5N), 8.58 (m, 2H, 2,6-C_5H_5N); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.12 (two $(CH_2)_{10}CH_3$), 22.69 (one of $(CH_2)_8$), 24.90 and 24.82 (CH_2CH_2COO), 31.92 – 28.13 (signals of $(CH_2)_8$ and C-1), 34.29 and 34.12 (CH_2COO), 67.68 – 65.18 (C-2 and C-3), 149.53, 138.51 and 124.73 (from C_5H_5N), 173.29 and 172.74 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 21.30; HR-MS *m/z*: 520.2246 $[M+H]^+$.

Monopyridinium salt of [2,3-bis(myristoyloxy)propyl]phosphonate (5b). White solid; m_p=78^{0}C, yield 91%, 299 mg; TLC R_f = 0.22 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 12,6 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.86 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_{12}CH_3$), 1.31-1.23 (m, 40H, 2x $(CH_2)_{10}CH_3$), 1.58 (m, 4H, 2x CH_2CH_2COO), 2.05 (dd, *J* = 18.5, 6.3 Hz, 2H, CH_2-1), 2.29 (m, 4 H, 2x CH_2COO), 4.12 (m, 1 H, one of CH_2-3), 4.41 (m, 1H, one of CH_2-3), 5.39 (m, 1H, H-2), 7.47 (m, 2H, 3,5-C_5H_5N), 7.89 (t, *J* = 7.5 Hz, 1H, 4-C_5H_5N), 8.58 (m, 2H, 2,6-C_5H_5N); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 14.12 (two $(CH_2)_{12}CH_3$), 22.69 (one of $(CH_2)_{10}$), 24.90 and 24.82 (CH_2CH_2COO), 31.92 – 28.33 (signals of $(CH_2)_{10}$ and C-1), 34.29 and 34.12 (CH_2COO), 67.82 – 65.06 (C-2 and C-3), 149.43, 138.51 and 124.73 (from C_5H_5N), 173.95 and 173.52 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 21.31; HR-MS *m/z*: 576.4864 $[M+H]^+$.

Monopyridinium salt of [2,3-bis(palmitoyloxy)propyl]phosphonate (5c). White solid; m_p=82^{0}C, yield 92%, 328 mg; TLC R_f = 0.23 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 12,7 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.86 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_{14}CH_3$), 1.34-1.19 (m, 48H, 2x $(CH_2)_{12}CH_3$), 1.63-1.52 (m, 4H, 2x CH_2CH_2COO), 2.05 (dd, *J* = 18.4, 6.8 Hz, 2H, CH_2-1), 2.28 (two t, *J* = 7.3 Hz, 4H, 2x CH_2COO), 4.16 (dd, *J* = 11.9, 7.0 Hz, one of CH_2-3), 4.36 (m, 1H, one of CH_2-3), 5.41 (m, 1H, H-2), 7.39 (m, 2H, 3,5-C_5H_5N), 7.80 (t, *J* = 7.5 Hz, 1H, 4-C_5H_5N), 8.54 (m, 2H, 2,6-C_5H_5N); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_{14}CH_3$), 22.58 (one of $(CH_2)_{12}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 27.69 (signals of $(CH_2)_{12}$ and C-1), 34.32 and 34.06 (CH_2COO), 68.08 – 65.06 (C-2 and C-3), 148.48, 137.41 and 124.37 (from C_5H_5N), 173.93 and 172.81 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 20.41; HR-MS *m/z*: 632.5473 $[M+H]^+$.

Monopyridinium salt of [2,3-bis(stearoyloxy)propyl]phosphonate (5d). White solid; m_p=82^{0}C, yield 90%, 346 mg; TLC R_f = 0.23 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 12,7 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.86 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_{16}CH_3$), 1.34-1.19 (m, 56H, 2x $(CH_2)_{14}CH_3$), 1.62-1.52 (m, 4H, 2x CH_2CH_2COO), 2.04 (dd, *J* = 18.5, 6.6 Hz, 2H, CH_2-1), 2.28 (two t, *J* = 7.3 Hz, 4H, 2x CH_2COO), 4.14 – 4.06 (m, 1H, one of CH_2-3), 4.34 (m, 1H, one of CH_2-3), 5.40 (m, 1H, H-2), 7.39 (m, 2H, 3,5-C_5H_5N), 7.80 (t, *J* = 7.5 Hz, 1H, 4-C_5H_5N), 8.54 (m, 2H, 2,6-C_5H_5N); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_{16}CH_3$), 22.58 (one of $(CH_2)_{12}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 28.37 (signals of $(CH_2)_{14}$ and C-1), 34.32 and 34.06 (CH_2COO), 68.18 – 64.98 (C-2 and C-3), 148.48, 137.41 and 124.37 (from C_5H_5N), 173.81 and 172.81 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 20.44; HR-MS *m/z*: 688.6244 $[M+H]^+$.

Monopyridinium salt [2,3-bis(oleoyloxy)propyl]phosphonate (5e). White colour, waxy material; yield 91%, 348 mg; TLC R_f = 0.20 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 12,7 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.86 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_7CH_3$), 1.33-1.20 (m, 40H, 20x CH_2), 1.64-1.53 (m, 4H, 2x CH_2CH_2COO), 2.07 (dd, *J* = 18.8, 6.5 Hz, 2H, CH_2-1), 2.30 (two t, *J* = 7.3 Hz, 4H, 2x CH_2COO), 4.15 – 4.04 (m, 1H, one of CH_2-3), 4.34 (m, 1H, one of CH_2-3), 5.41 – 5.25 (m, 5H, H-2 and 2x -CH=CH-), 7.39 (m, 2H, 3,5-C_5H_5N), 7.80 (t, *J* = 7.5 Hz, 1H, 4-C_5H_5N), 8.54 (m, 2H, 2,6-C_5H_5N); ^{13}C NMR (151 MHz,

$CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_7CH_3$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 28.34 (signals of $(CH_2)_{14}$ and C-1), 34.30 and 34.12 (CH_2COO), 68.18 – 65.13 (C-2 and C-3), 130.04 and 129.72 (CH=CH), 148.48, 137.41 and 124.37 (from C_5H_5N), 173.24 and 172.73 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 20.12; HR-MS *m/z*: 684.6063 $[M+H]^+$.

Monopyridinium salt [2,3-bis(linoleoyloxy)propyl]phosphonate (5f). White colour waxy material; yield 88%, 335mg; TLC R_f = 0.18 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 12,6 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.88 (two t, *J* = 7.0 Hz, 6H, 2x $(CH_2)_4CH_3$), 1.33-1.20 (m, 24H, 12x CH_2), 1.68-1.58 (m, 4H, 2x CH_2CH_2COO), 2.21 – 1.97 (m, 10H, CH_2-1 and 4x $=CHCH_2$), 2.35 – 2.23 (m, 4H, 2x CH_2COO), 4.16 (m, 1H, one of CH_2-3), 4.37 (m, 1H, one of CH_2-3), 5.44 – 5.23 (m, 9H, H-2 and 4x -CH=CH-), 7.54 (m, 2H, 3,5-C_5H_5N), 8.08 (m, *J* = 7.5 Hz, 1H, 4-C_5H_5N), 8.67 (m, 2H, 2,6-C_5H_5N); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_4CH_3$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 28.37 (signals of $(CH_2)_{10}$ and C-1), 34.30 and 34.12 (CH_2COO), 68.18 – 64.13 (C-2 and C-3), 130.25 – 127.91 (signals of CH=CH), 148.48, 137.41 and 124.37 (from C_5H_5N), 173.24 and 172.73 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 21.64; HR-MS *m/z*: 680.5463 $[M+H]^+$.

Monopyridinium salt of [2,3-bis(conjugated linoleoyloxy)propyl]phosphonate (5g). White colour waxy material; yield 92%, 350 mg; TLC R_f = 0.18 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 12,6 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.92 – 0.82 (m, 6H, 2x $(CH_2)_4CH_3$), 1.42 – 1.22 (m, 32H, 16x CH_2), 1.72 – 1.64 (m, 8H, 2x CH_2CH_2COO and 2x $=CHCH_2CH_2$), 8.70 (m, 2H, 2,6-C_5H_5N), 2.18 – 2.06 (m, 10H, CH_2-1 and 4x $=CHCH_2$), 2.31 – 2.26 (m, 4H, 2x CH_2COO), 4.19 – 4.11 (m, 1H, one of CH_2-3), 4.42 – 4.37 (m, 1H, one of CH_2-3), 5.37 – 5.24 (m, 3H, H-2, 2x one of =CH; H-9' and H-13"), 5.66 – 5.60 (m, 2H, 2x one of =CH; H-12' and H-10"), 6.02 – 5.85 (m, 2H, 2x one of =CH; H-10' and H-12"), 6.32 – 6.23 (m, 2H, H-11' and H-11"), 7.62 (m, 2H, 3,5-C_5H_5N), 8.12 (t, *J* = 7.5 Hz, 1H, 4-C_5H_5N); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.80 and 13.79 (two $(CH_2)_4CH_3$), 33.99 – 22.44 (signals of $(CH_2)_{24}$ of 9*c*,11*t*-CLA and 10*t*,12*c*-CLA and C-1), 67.61 – 64.88 (C-2 and C-3), 125.30 (from C_5H_5N), 125.53 (C-11'), 125.60 (C-11"), 128.55 (C-12"), 128.70 (C-10'), 129.65 (C-9'), 129.95 (C-13"), 134.39 (C-10"), 134.64 (C-12'), 145.80 and 141.05 (from C_5H_5N) 173.87 and 173.78 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v $_3$) δ: 22.27; HR-MS *m/z*: 680.5438 $[M+H]^+$.

(') – signals from (9*c*, 11*t*)-conjugated linoleic acid (CLA)

(") - signals from (10*t*, 12*c*)-conjugated linoleic acid

Synthesis of [2,3-bis(acyloxy)propyl]phosphonocholine (6a-g)

To a suspension of the monopyridinium salt of phosphonic acid (**5a-g**) (0,27 mmol), choline tosylate (147 mg, 0,53 mmol) in mixture of dry pyridine:CCl_3CN (10 mL, 80:20 v/v) DCC (165 mg, 0,80 mmol) was added at room temperature and the reaction was stirred for 48 h under nitrogen. Then the solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel (eluent: $CHCl_3$:MeOH:H_2O 65:25:3 v/v/v). The spectral data of obtained compounds **6a-g** are given below:

[2,3-Bis(lauroyloxy)propyl]phosphonocholine (6a). White solid; m_p=33^{0}C, yield 68%, 112 mg; TLC R_f = 0.41 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 15,9 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.86 (two t, *J* = 6.6 Hz, 6H, 2x $(CH_2)_{10}CH_3$), 1.31 – 1.22 (m, 32H, 2x $(CH_2)_8CH_3$), 1.61-1.50 (m, 4H, 2x CH_2CH_2COO), 2.02 – 1.97 (m, 2H, CH_2-1), 2.26 (two t, *J* = 7.5 Hz, 4H, 2x CH_2COO), 3.16 (s, 9H, $N(CH_3)_3$), 3.44 – 3.36 (m, 2H, CH_2-$N(CH_3)_3$), 4.08 (m, 1H, one of CH_2-3), 4.22 – 4.16 (m, 2H, P-O-CH_2), 4.28 (m, 1H, one of CH_2-3), 5.34 (m, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_{10}CH_3$), 22.58 (one of $(CH_2)_{10}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 27.78 (signals of $(CH_2)_8$ and C-1), 34.32 and 34.06 (CH_2COO), 54.18 (-$N(CH_3)_3$), 59.56 (P-O-CH_2), 68.18 – 65.03 (C-2, C-3 and CH_2-$N(CH_3)_3$), 173.81 and 172.81 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 20.04; HR-MS *m/z*: 605.8217 $[M+H]^+$.

[2,3-Bis(myristoyloxy)propyl]phosphonocholine (6b). White solid; m_p=46^{0}C, yield 70%, 126 mg; TLC R_f = 0.42 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 15,8 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.86 (two t, *J* = 6.6 Hz, 6H, 2x $(CH_2)_{12}CH_3$), 1.34 – 1.22 (m, 40H, 2x $(CH_2)_{10}CH_3$), 1.59-1.50 (m, 4H, 2x CH_2CH_2COO), 2.02 – 1.97 (m, 2H, CH_2-1), 5.32 (m, 1H, H-2), 2.28 (two t, *J* = 7.5 Hz, 4H, 2x CH_2COO), 3.16 (s, 9H, $N(CH_3)_3$), 3.50 – 3.46 (m, 2H, CH_2-$N(CH_3)_3$), 4.10 (m, 1H, one of CH_2-3), 4.22 – 4.16 (m, 2H, P-O-CH_2), 4.33 – 4.24 (m, 1H, one of CH_2-3); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_{12}CH_3$), 22.58 (one of $(CH_2)_{10}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 28.14 (signals of $(CH_2)_{12}$ and C-1), 34.32 and 34.06 (CH_2COO), 54.18 (-$N(CH_3)_3$), 59.56 (P-O-CH_2), 68.18 – 65.03 (C-2, C-3 and CH_2-$N(CH_3)_3$), 173.81 and 172.81 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 20.12; HR-MS *m/z*: 661.9257 $[M+H]^+$.

[2,3-Bis(palmitoyloxy)propyl]phosphonocholine (6c). White solid; m_p=62^{0}C, yield 67%, 130 mg; TLC R_f = 0.41 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 15,9 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.84 (two t, *J* = 6.6 Hz, 6H, 2x $(CH_2)_{14}CH_3$), 1.34-1.11 (m, 48H, 2x $(CH_2)_{12}CH_3$), 1.62-1.48 (m, 4H, 2x CH_2CH_2COO), 1.98 – 1.89 (m, 2H, CH_2-1), 2.26 (two t, *J* = 7.5 Hz, 4H, 2x CH_2COO), 3.16 (s, 9H, $N(CH_3)_3$), 3.50 – 3.46 (m, 2H, CH_2-$N(CH_3)_3$), 4.10 (m, 1H, one of CH_2-3), 4.22 – 4.16 (m, 2H, P-O-CH_2), 4.31 – 4.21 (m, 1H, one of CH_2-3), 5.32 (m, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_{14}CH_3$), 22.58 (one of $(CH_2)_{12}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 28.34 (signals of $(CH_2)_{16}$ and C-1), 34.32 and 34.06 (CH_2COO), 54.18 (-$N(CH_3)_3$), 59.56 (P-O-CH_2), 68.18 – 65.03 (C-2, C-3 and CH_2-$N(CH_3)_3$), 173.81 and 172.81 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 19.68; HR-MS *m/z*: 718.0068 $[M+H]^+$.

[2,3-Bis(stearoyloxy)propyl]phosphonocholine (6d). White solid, m_p=70^{0}C, yield 72%, 151 mg; TLC R_f = 0.41 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 15,9 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.84 (two t, *J* = 6.6 Hz, 6H, 2x $(CH_2)_{16}CH_3$), 1.34-1.11 (m, 56H, 2x $(CH_2)_{14}CH_3$), 1.63-1.48 (m, 4H, 2x CH_2CH_2COO), 1.94 (dd, *J* = 18.2, 6.8 Hz, 2H, CH_2-1), 2.27 (two t, *J* = 7.5 Hz, 4H, 2x CH_2COO), 3.18 (s, 9H, $N(CH_3)_3$), 3.61 – 3.50 (m, 2H, CH_2-$N(CH_3)_3$), 4.11 (dd, *J* = 12.0, 7.5 Hz, 1H, one of CH_2-3), 4.29 – 4.20 (m, 2H, P-O-CH_2), 4.51 – 4.41 (m, 1H, one of CH_2-3), 5.27 (m, 1H, H-2); ^{13}C NMR (151 MHz,

$CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_{16}CH_3$), 22.58 (one of $(CH_2)_{16}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 28.24 (signals of $(CH_2)_{16}$ and C-1), 34.32 and 34.06 (CH_2COO), 54.18 ($-N(CH_3)_3$), 59.56 (P-O-CH_2), 68.16 – 64.98 (C-2, C-3 and CH_2-$N(CH_3)_3$), 173.81 and 172.81 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 19.57; HR-MS *m/z*: 774.1163 $[M+H]^+$.

[2,3-Bis(oleoyloxy)propyl]phosphonocholine (6e). Colourless oil, yield 68%, 142 mg; TLC R_f = 0.41 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 15,9 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.84 (two t, *J* = 6.4 Hz, 6H, 2x $(CH_2)_7CH_3$), 1.32-1.10 (m, 40H, 20x -CH_2), 1.61-1.49 (m, 4H, 2x CH_2CH_2COO), 1.95 (dd, *J* = 18.2, 6.8 Hz, 2H, CH_2-1), 2.25 (two t, *J* = 7.6 Hz, 4H, 2x CH_2COO), 3.19 (s, 9H, $N(CH_3)_3$), 3.61 – 3.50 (m, 2H, CH_2-$N(CH_3)_3$), 4.11 (dd, *J* = 11.9, 7.4 Hz, 1H, one of CH_2-3), 4.29 – 4.20 (m, 2H, P-O-CH_2), 4.45 (m, 1H, one of CH_2-3), 5.27 (m, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_7CH_3$); 22.58 (one of $(CH_2)_{14}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 28.14 (signals of $(CH_2)_{14}$ and C-1), 34.32 and 34.06 (CH_2COO), 54.18 ($-N(CH_3)_3$), 59.56 (P-O-CH_2), 68.20 – 64.38 (C-2, C-3 and CH_2-$N(CH_3)_3$), 130.04 and 129.72 (CH=CH), 173.81 and 172.81 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 19.34; HR-MS *m/z*: 770.1076 $[M+H]^+$.

[2,3-Bis(linoleoyloxy)propyl]phosphonocholine (6f). Colourless oil; yield 60%, 125 mg; TLC R_f = 0.41 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 15,9 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.86 (two t, *J* = 6.8 Hz, 6H, 2x $(CH_2)_4CH_3$), 1.29-1.14 (m, 24H, 12x -CH_2), 1.61-1.49 (m, 4H, 2x CH_2CH_2COO), 2.21 – 1.95 (m, 10H, CH_2-1 and 4x =$CHCH_2$), 2.25 (two t, *J* = 7.6 Hz, 4H, 2x CH_2COO), 3.18 (s, 9H, $N(CH_3)_3$), 3.62 – 3.53 (m, 2H, CH_2-$N(CH_3)_3$), 4.07 (m, 1H, one of CH_2-3), 4.32 – 4.22 (m, 2H, P-O-CH_2), 4.47 (m, 1H, one of CH_2-3), 5.39 – 5.20 (m, 9H, H-2 and 4x -CH=CH-); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_4CH_3$), 22.58 (one of $(CH_2)_{14}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 28.12 (signals of $(CH_2)_{10}$ and C-1), 34.30 and 34.10 (CH_2COO), 54.18 ($-N(CH_3)_3$), 59.56 (P-O-CH_2), 68.18 – 65.12 (C-2, C-3 and CH_2-$N(CH_3)_3$), 130.30 – 127.87 (signals of CH=CH 173.81 and 172.81 (C(O)O),); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 18.89; HR-MS *m/z*: 766.5843 $[M+H]^+$.

[2,3-Bis(conjugated linoleoyloxy)propyl]phosphonocholine (6g). Colourless oil; yield 65%, 135 mg; TLC R_f = 0.41 ($CHCl_3$:MeOH:H_2O 65:25:2 v/v/v), HPLC: Betasil DIOL, R_t= 15,9 min; ^{1}H NMR (600 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 0.86 (two t, *J* = 6.8 Hz, 6H, 2x $(CH_2)_4CH_3$), 1.29-1.14 (m, 32H, 16x -CH_2), 1.61-1.49 (m, 8H, 2x CH_2CH_2COO and 2x =$CHCH_2CH_2$), 2.21 – 1.95 (m, 10H, CH_2-1 and 4x =$CHCH_2$), 2.25 (two t, *J* = 7.6 Hz, 4H, 2x CH_2COO), 3.15 (s, 9H, $N(CH_3)_3$), 3.60 – 3.43 (m, 2H, CH_2-$N(CH_3)_3$), 4.07 (m, 1H, one of CH_2-3), 4.42 – 4.25 (m, 3H, one of CH_2-3 and P-O-CH_2), 5.37 – 5.24 (m, 3H, H-2, 2x one of =CH; H-9' and H-13"), 5.65 (m, 2H, 2x one of =CH; H-12' and H-10"), 5.94 (m, 2H, 2x one of =CH; H-10' and H-12"), 6.32 – 6.24 (m, 2H, H-11' or H-11"); ^{13}C NMR (151 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 13.85 (two $(CH_2)_4CH_3$), 22.58 (one of $(CH_2)_{14}$), 24.90 and 24.81 (CH_2CH_2COO), 31.84 – 27.54 (signals of $(CH_2)_{10}$ and C-1), 34.30 and 34.10 (CH_2COO), 54.18 ($-N(CH_3)_3$), 59.56 (P-O-CH_2), 68.18 – 65.07 (C-2, C-3 and CH_2-$N(CH_3)_3$), 125.56 (C-11'), 125.68 (C-11"), 128.57 (C-12"), 128.71 (C-

10'), 129.88 (C-9'), 130.19 (C-13"), 134.57 (C-10"), 134.83 (C-12'), 173.81 and 172.81 (C(O)O); ^{31}P NMR (243 MHz, $CDCl_3$:MeOD 2:1 v/v) δ: 19.67; HR-MS *m/z*: 766.4877 $[M+H]^+$.
(') – signals from (9*c*, 11*t*)-conjugated linoleic acid (CLA)
(") - signals from (10*t*, 12*c*)-conjugated linoleic acid

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