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Full Paper

Phosphatidylcholine with *cis*-9,*trans*-11 and *trans*-10,*cis*-12 Conjugated Linoleic Acid Isomers: Synthesis and Cytotoxic Studies

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Novel phosphatidylcholines and lysophosphatidylcholines with *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid (CLA) were synthesized in high yields (75–99 %). The in vitro cytotoxic activities of these compounds against three human cancer cell lines (HL-60, MCF-7, and HT-29) were evaluated. The results revealed that there are differences in the activity between phosphatidylcholine with *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA acyl groups. 1,2-Di(9*Z*,11*E*)-octadecadienoyl-*sn*-glycero-3-phosphocholine was the most potent cytotoxic agent among all tested CLA derivatives and its IC_{50} (concentration of a compound that inhibits the proliferation of 50 % of the cancer cell population) was 29.4 μM against HL-60. Moreover, phosphatidylcholines with CLA acyls exhibited much lower cytotoxicity against non-cancer cells (Balb/3T3) than free CLA isomers.

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Introduction

For more than 25 years, conjugated linoleic acid (CLA) has been of interest to researchers owing to its many biological activities. It has been proved that it prevents cholesterol-induced atherosclerosis in animal models,[1] enhances growth promotion by increasing body protein,[2,3] exhibits antiobesity properties (reviewed by Whigham et al.),[4] and inhibits carcinogenesis.[5,6] More recent studies have shown that CLA can also interact synergistically with other anticancer agents and enhance their effects.[7,8] CLA (an unsaturated conjugated fatty acid with two double bonds) can exist in several positional (from C-7 up to C-14) and geometrical (*trans*, *cis*) isomers. The most common is *cis*-9,*trans*-11, which occurs in the meat and milk of ruminants.[9,10] Another well-known isomer with respect to bioactivity is *trans*-10,*cis*-12. It is formed together with *cis*-9, *trans*-11 as the second major product of the alkaline isomerization of linoleic acid.[11] It has been observed that a difference in molecular structure of particular isomers can cause variation in their activity. For instance, the *cis*-9,*cis*-11 isomer showed the ability to inhibit more than 60 % of the breast cancer MCF-7 cell population, whereas *cis*-9,*trans*-11 CLA inhibited only 20 %.[12] The opposite effect was noticed in the colon cancer cell line HT-29, where the highest cytotoxicity was observed for the *cis*-9,*trans*-11 isomer.[13] The isomer that exhibits the widest inhibitory effect is *trans*-10,*cis*-12. It strongly reduces proliferation of breast cancer (MCF-7),[12] rat hepatoma (dRLh-84),[14] and colon cancer (Caco-2, HT-29, MIP-101) cells.[13,15] Also *trans*,*trans* isomers of CLA act strongly against breast cancer[16] and osteosarcoma.[17] It is apparent from these reports that it is important to test separately newly isolated or synthesized CLA isomers or its derivatives.

Many chemotherapeutics such as cisplatin and 5-fluorouracil are effective in cancer therapy, but some side effects can occur during treatment.[18] A modern anticancer drug is required to be highly bioactive and specific against cancer cells only.[19] It should also be easily absorbed from the intestinal tract when administered orally. CLA in the form of a free fatty acid may have limited absorption capacity from the intestine as suggested similarly for polyunsaturated fatty acids in research carried out by Carineli et al.[20] CLA coupled with a polar molecule such as phosphatidylcholine (PC) should have favourable bioavailability in comparison with CLA itself.

Recently, we presented work on the synthesis of PC with a mixture of predominantly *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers in the *sn*-1 and *sn*-2 position.[21] We also showed the results of a cytotoxic study on breast cancer cells and promyelocytic leukaemia. In the current paper, we present an extension of this earlier research. New PCs and lysophosphatidylcholines were prepared with the individual isomers *cis*-9, *trans*-11 and *trans*-10,*cis*-12. The cytotoxic activity of the synthesized compounds was also tested.

Results and Discussion

A common procedure to obtain PC[22,23] with the same acyl residue in the *sn*-1 and *sn*-2 position involves using the cadmium salt of *sn*-glycero-3-phosphocholine (GPC) as a starting material and was applied to synthesis of 1,2-di-(9*Z*,11*E*)-octadecadienoyl-*sn*-glycero-3-phosphocholine (**2d**) and 1,2-di-(10*E*,12*Z*)-octadecadienoyl-*sn*-glycero-3-phosphocholine (**2e**). The synthesis of 1,2-di-(9*Z*,12*Z*)-octadecadienoyl-*sn*-glycero-3-phosphocholine (**2b**) and 1,2-di(conjugated)linoleoyl-*sn*-glycero-3-phosphocholine (**2c**) was described earlier.[21] 1,2-Octadecanoyl-*sn*-glycero-3-phosphocholine (**2a**)[24] was also prepared to compare its cytotoxic activity with those of **2b**–**e**. Fatty acids used for the synthesis of PCs and the structure of one example of a synthesis product (**2a**) are shown in Fig. 1.

Acylation with *cis*-9,*trans*-11 (**1d**) and *trans*-10,*cis*-12 (**1e**) CLA isomers in a biphasic reaction was conducted in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) and a coupling agent *N*,*N*′-dicyclohexylcarbodiimide (DCC) (Scheme 1). A detailed description of the reaction conditions has been given previously.[21] Pure isomers **1d** and **1e** used in the synthesis were purified from the alkali-isomerized linoleic acid according to methods we published elsewhere.[11,25] The PCs were obtained in good yields of 96 % (**2a**), 75 % (**2d**), and 75 % (**2e**). The isomer composition of CLA residues in **2d** and **2e** obtained by gas chromatography (GC) was exactly the same as for the CLA isomers **1d** and **1e** that were used in the synthesis. The *cis*-9, *trans*-11 CLA isomer constituted 94.2 % of the acyl residues in **2d**. A trace of *trans*-10,*cis*-12 (2.5 %) and 3.3 % of other isomers (mainly *trans*,*trans* and *cis*,*cis*) were also present. The *trans*-10, *cis*-12 isomer constituted 96.5 % of the acyl residues in **2e**. The *cis*-9,*trans*-11 isomer was presented only in 2.8 %. The content of *trans*,*trans* and *cis*,*cis* isomers did not exceed 0.8 %.

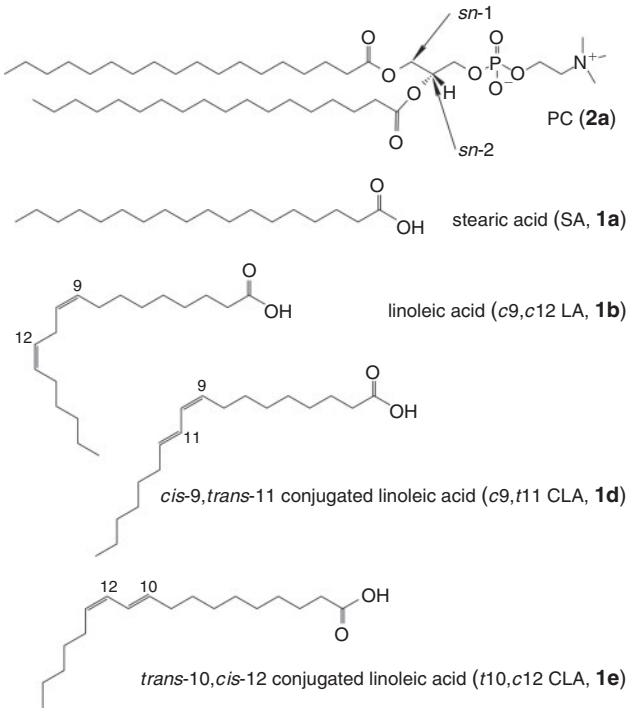
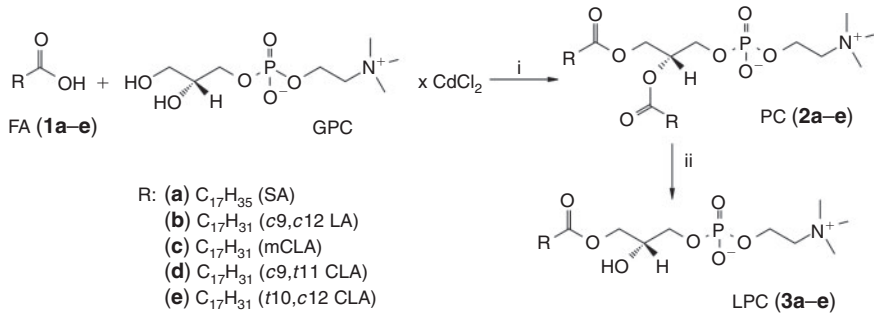


Fig. 1. Fatty acids used in the synthesis of phosphatidylcholines. Structure of a synthesis product example.

The second group of PCs obtained contained different fatty acid moieties in the *sn*-1 and *sn*-2 positions. The synthesis of this type of phospholipids is a challenging task requiring regioselective incorporation of different fatty acid residues in two positions of the GPC backbone. The literature contains several useful methods for the synthesis of mixed-chain phosphatidylcholines (MC-PC). Most of them were reviewed by D'Arrigo et al.[26] Two of them drew our attention. The first method, based on the tin-mediated synthesis of lysophosphatidylcholine (Scheme 2b), involved the formation of a stannylene derivative, and subsequent acylation with a stoichiometric amount of acyl chloride to afford 1-acyl-2-hydroxy-*sn*-glycero-3-phosphocholine (1-acyl LPC), followed by a second acylation in the *sn*-2 position.[27,28] This method is useful for the synthesis of 1-acyl LPC using medium-chain acyl chlorides, which are commercially available. However, the synthesis of 1-acyl LPC with conjugated linoleoyl residues requires an additional step of acid chloride synthesis. We attempted to obtain *trans*-10, *cis*-12 conjugated linoleoyl chloride using oxalyl chloride (Scheme 2a), which tends to be a milder, more selective reagent than thionyl chloride. The acid chloride was prepared according to the method described by Mattson and Volpenhein.[29] One part of the fatty acid was mixed with 1.2 parts of oxalyl chloride on a weight basis. The reaction was carried out for 48 h. This method afforded the acid chloride in yield of 88 % (according to ^{1}H NMR). Subsequent synthesis of 1-(10*E*,12*Z*)-octadecadienoyl-2-hydroxy-*sn*-glycero-3-phosphocholine via the stannylene derivative was effective. Product formation in 80–85 % yield after 30 min of reaction was observed by HPLC. Unfortunately, washing of 1-acyl LPC in water/alcohol solution with heptane was an inefficient method of removing reaction by-products. The water/alcohol extract contained triethylamine chloride, which was difficult to separate from



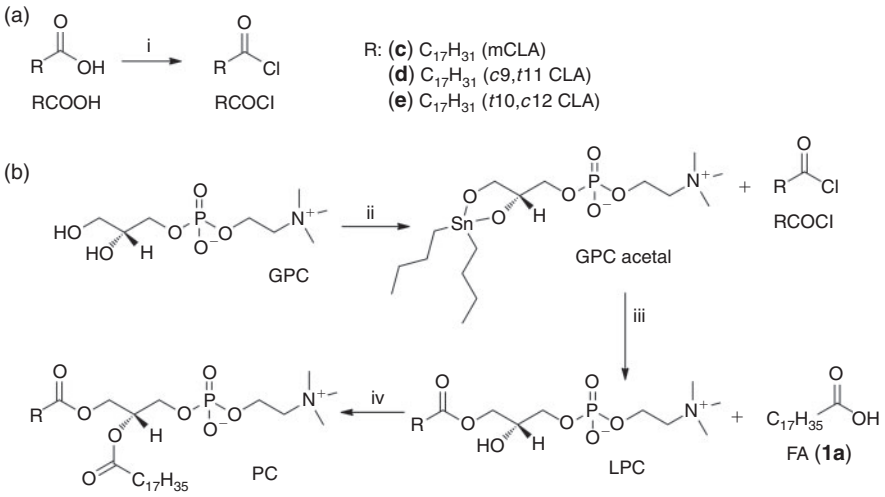
Scheme 1. Reagents and conditions: (i) Dimethylaminopyridine (DMAP), *N*,*N*′-dicyclohexylcarbodiimide (DCC), anhydrous CH_2Cl_2, room temperature, 40 h, **2a**–**e** (75–96 %); (ii) phospholipase A_2 (PLA_2), Tris-HCl (pH 8.5, 0.1 M, 0.75 mM $CaCl_2$), sodium docusate (AOT), iso-octane, 40°C, 30 min, **3a**–**e** (89–99 %).

lysophosphatidylcholine in both the crystallization process with cold acetone as well as by column chromatography.

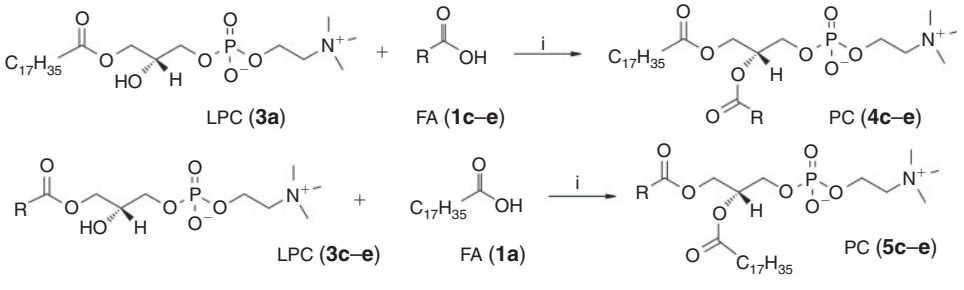
This led us to the conclusion that the aforementioned method is not suitable for the synthesis and extraction of 1-acyl LPC with long fatty acyl residues. C-18 fatty acyl residues have higher hydrophobicity than medium- and short-chain LPCs, resulting in large losses during extraction with non-polar heptane or hexane. Therefore, we focussed on an alternative route that consists of the enzymatic hydrolysis of 1,2-diacyl-*sn*-glycero-3-phosphocholine in sodium docusate (AOT) mixed reversed micelles catalyzed by phospholipase A_2 (PLA_2)[30] (Scheme 1) and subsequent re-esterification with the appropriate fatty acid using DCC and DMAP (Scheme 3).

1-Acyl LPC is a more common intermediate in PC synthesis than 2-acyl LPC.[28,31,32] It is more stable in the reaction mixture and does not significantly undergo acyl migration from *sn*-1 to *sn*-2, which is favoured in commonly used organic solvents.[33,34] Hydrolysis of **2a**–**e** afforded 1-acyl LPCs **3a**–**e** in excellent yield (89–99 %) and in a short time (30 min). The MC-PCs were prepared as two groups of compounds. First were PCs with a stearoyl residue at *sn*-1 and conjugated linoleoyl isomers at *sn*-2. Acylation of **3a** by **1c**, **1d** or **1e** gave three products **4c**, **4d**, and **4e** in 79, 75, and 73 % yield respectively. The second group were PCs with conjugated linoleoyl isomer residues in the *sn*-1 and stearoyl in the *sn*-2 positions. They were obtained in 80 % (**5c**), 80 % (**5d**), and 82 % (**5e**) yield by acylation of **3c**, **3d**, and **3e** with stearic acid (**1a**).

Positional analysis of the mixed-chain PC was carried out to verify fatty acid distribution in the mixed-chain PC obtained. The analysis was based on the same method of PC hydrolysis that was used during the preparation of 1-acyl LPC.[30] In the present case, the hydrolysis was scaled down according to the method described earlier.[21] The PLA_2 used in this reaction is a selective enzyme that hydrolyzes the ester bond only in the *sn*-2 position, which allows the determination of the fatty acid composition at each position separately. The positional analysis of the acyl residues in **4c**–**e** showed that the percentage of CLA isomers in the *sn*-1 position was 7.4–12.1 %, whereas at *sn*-2, it was 86.4–89.6 %. Similarly, CLA was present in both the *sn*-1 (86.7–90.9 %) and the *sn*-2 (9.0–12.6 %) position of **5c**–**e** CLA (Table 1). Our previous studies showed that no non-enzymatic hydrolysis or migration processes took place during the PLA_2-catalyzed hydrolysis of PC in mixed reversed micelles.[35] This indicates that partial acyl migration from *sn*-1 to *sn*-2 occurred during the purification of **3a**, **3c**–**d**. 1-Acyl LPC is much more stable than 2-acyl LPC and the equilibrium concentration ratio between these two compounds is ~9 : 1.[35,36] Therefore, some traces of 2-acyl LPC always occur in 1-acyl LPC preparations used in the synthesis of **4c**–**e**, **5c**–**e**.



Scheme 2. (a) Synthesis of fatty acid chloride; and (b) synthesis of mixed-chain phosphatidylcholine via stannylene derivatives. Reagent and conditions: (i) $(COCl)_2$, hexane, room temperature (rt), 1 h; (ii) dibutyltin oxide (DBTO), 2-propanol, bp, 1 h; (iii) triethylamine (TEA), rt, 1 h; (iv) DMAP, DCC, anhydrous CH_2Cl_2, rt, 40 h.



Scheme 3. Reagents and conditions: (i) DMAP, DCC, anhydrous CH_2Cl_2, room temperature, 72 h, **4c**–**e** (75–79 %); **5c**–**e** (80–82 %).

All the synthesized compounds were characterized by ^{1}H, ^{13}C, ^{31}P NMR, and high-resolution mass spectrometry (HRMS). Four signals characteristic of the *cis*-9,*trans*-11 and *trans*-10, *cis*-12 CLA moiety olefinic protons of **2c**–**e**, **3c**–**e**, **4c**–**d**, and **5c**–**d** were identified in the ^{1}H NMR at δ 6.28–6.29 for the inner *trans*, 5.92–5.95 for the inner *cis*, 5.62–5.65 for the outer *trans*, and 5.29–5.31 ppm for the outer *cis* olefinic protons. The carbon atoms of the conjugated double bond systems in the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers gave four signals in the ^{13}C NMR spectrum of **2c**–**e**, **3c**–**e**, **4c**–**e**, and **5c**–**e** in the range 135–125 ppm. Detailed assignments are given in the experimental section. Two signals from the carboxylic carbon of the ester groups in the *sn*-2 (δ 174.72–174.73) and *sn*-1 (δ 173.33–173.35) positions were present in the ^{13}C NMR spectra of 1,2-diacyl-*sn*-glycero-3-phosphocholines. They were shifted upfield in comparison with the corresponding carbon atom signals in the CLA ^{13}C NMR spectrum (δ 179.72). Signals from carboxylic carbons (δ 174.06–174.13) in the *sn*-1 position of 1-acyl-LPCs (**3a**–**e**) were shifted downfield compared with the corresponding signals in PCs.

Table 1. Fatty acid composition of mixed-chain phosphatidylcholine (4c–e and 5c–e)

SA, stearic acid; CLA, conjugated linoleic acid; mCLA, mixture of conjugated linoleic acid isomers

Compound	Fatty acid	Composition [%]	
		sn-1	*sn*-2
4c	SA	92.6	10.8
	mCLA[A]	7.4	89.2
4d	SA	89.0	13.6
	*c*9,*t*11 CLA[B]	11.0	86.4
4e	SA	87.9	11.4
	*t*10,*c*12 CLA[C]	12.1	89.6
5c	SA	13.3	88.7
	mCLA[A]	86.7	11.3
5d	SA	9.5	91.0
	*c*9,*t*11 CLA[B]	90.9	9.0
5e	SA	10.6	87.3
	*t*10,*c*12 CLA[C]	89.4	12.6

[A]Mixture of CLA isomers: 48.5 % *cis*-9,*trans*-11, 48.3 % *trans*-10,*cis*-12, 2.5 % other CLA isomers.
[B]94.2 % *cis*-9,*trans*-11, 5.8 % other CLA isomers.
[C]96.5 % *trans*-10,*cis*-12, 3.5 % other CLA isomers.

Cytotoxic Study

The cytotoxic activity of the synthesized PCs **2d**–**e**, **4c**–**d**, and **5c**–**d** and LPCs **3c**–**e** with CLA isomers residues was compared with the activity of PCs containing commonly occurring stearic (SA) and linoleic acid (LA) in their structures (**2a**, **2b**, **3a**, **3b**) and with the free fatty acids (**1a**–**e**) used for the synthesis. Cisplatin was used as a positive control. Antitumour activities were evaluated in vitro against three human cancer cell lines: human promyelocytic leukaemia (HL-60), breast cancer (MCF-7), and colon cancer (HT-29). The mouse fibroblast cell line Balb/3T3 was used in the experiment to investigate the effect of the tested compounds on non-cancer cells. The results of the in vitro cytotoxic tests were calculated as an IC_{50} (concentration of a compound that inhibits the proliferation of 50 % of the cancer cell population) (mg mL^{-1}) and converted to micromolar (μmol mL^{-1}). As shown in Table 2, the fatty acids (**1a**–**e**) showed medium cytotoxicity to both cancer and non-cancer (Balb/3T3) cell lines.

Isomer *trans*-10,*cis*-12 CLA (**1e**) exhibited higher inhibitory activity against HL-60 (35.8 μM), MCF-7 (110.1 μM), and HT-29 (103.3 μM) than *cis*-9,*trans*-11 CLA (**1d**), linoleic acid (**1b**), and stearic acid (**1a**). The lowest cytotoxic effect towards HL-60 and MCF-7 cell lines was observed for SA (**1a**). This suggests that the cytotoxicity of the fatty acid (FA) is associated with the presence of double bonds in the FA chain. Generally, activity is higher if double bonds are conjugated. These results are consistent with our previous studies[21] and those obtained by others.[17,37]

Among all tested tumour cell lines, the HL-60 line was the most sensitive to PC compounds (**2a**–**e**, **4c**–**e**, **5c**–**e**) (Fig. 2, Table 3). Among the PCs with the same fatty acid in the *sn*-1 and *sn*-2 position, PC with the *cis*-9,*trans*-11 CLA moiety (**2d**) showed better activity against HL-60 (29.4 μM) and MCF-7 (45.8 μM) than PC with the *trans*-10,*cis*-12 CLA moiety (**2e**). Surprisingly, the more potent *trans*-10,*cis*-12 isomer is less active against HL-60 and MCF-7 as an acyl residue in the PC molecules (**2e**) than the free acid (**1e**). Moreover, PCs with the *trans*-10,*cis*-12 CLA isomer (**2e**, **4e**, **5e**) reduced proliferation of HT-29 cells more strongly than PCs with *cis*-9,*trans*-11 CLA moieties (**2d**, **4e**, **5e**) (Fig. 2).

Mixed-chain PCs (**4c**–**e**, **5c**–**e**) were synthesized in order to determine whether the position of the CLA isomer in the PC molecule is related to their activity. All of the mixed-chain PCs (**4c**–**e**, **5c**–**e**) showed lower inhibitory activities against cancer cells than the other tested compounds (Fig. 2, Table 3).

Table 2. Anti-proliferative in vitro activity of fatty acid on selected cell lines

CLA, conjugated linoleic acid

Compound label	Fatty acid	Compound IC_{50}^{A} [μM]			
		HL-60	MCF-7	HT-29	Balb/3T3
1a	Stearic acid (SA)	120.4 ± 9.6	>351.5	110.9 ± 4.4	138.6 ± 13.3
1b	Linoleic acid (LA)	102.6 ± 13.4	253.2 ± 12.8[B]	115.0 ± 12.6	116.0 ± 6.7[B]
1c	*cis*-9,*trans*-11 + *trans*-10,*cis*-12 CLA	65.7 ± 19.3[B]	124.9 ± 2.1[B]	134.8 ± 9.1	125.4 ± 3.7[B]
1d	*cis*-9,*trans*-11 CLA	102.4 ± 9.9	112.4 ± 6.2	117.9 ± 1.2	121.1 ± 1.7
1e	*trans*-10,*cis*-12 CLA	35.8 ± 28.1	110.1 ± 4.8	103.3 ± 22.7	100.4 ± 12.3
	Cisplatin	0.8 ± 0.3[B]	14.1 ± 1.0[B]	14.3 ± 12.0	9.1 ± 2.2[C]

[A]The concentration of a compound that inhibits the proliferation of 50 % of the cancer cell population. Data are presented as mean ± s.d. of three independent experiments.
[B]Data derived from separate experiment.[21]
[C]Data derived from separate experiment.[38]

The inhibitory activities of PC with CLA isomer residues at *sn*-2 and stearoyl at *sn*-1 (**4c**–**e)** and PC with CLA isomer residues at *sn*-1 and stearoyl at *sn*-2 (**5c**–**e)** were not significantly different to each other towards HL-60 and MCF-7. The HT-29 line showed greater sensitivity to mixed-chain PC with *trans*-10, *cis*-12 (**4e**, **4d**) than *cis*-9,*trans*-11 CLA (**5e**, **5d**) residues. Proliferation inhibition by **4e** and **5e** was found to be 15 and 19 % respectively whereas **4d** and **5d** showed no inhibition for HT-29 (Fig. 2). No significant difference in biological activity between pairs **4c** and **5c**, **4d** and **5d**, and **4e** and **5e** of mixed-chain PCs with CLA residues in *sn*-1 and *sn*-2 positions was observed. Lysophosphatidylcholines **3a**–**c** showed better cytotoxicity against HL-60 than PCs. 1-Acyl LPC with a stearoyl residue (**3a**) showed unexpected activity (59.1–60.4 μM) towards all cancer lines but also healthy Balb/3T3 cells. It is noteworthy that all PC derivatives exhibited a much lower cytotoxicity against healthy cells (Balb/3T3) than free FA (Tables 2 and 3). The best activity among lysophosphatidylcholines with conjugated linoleic acid was observed for the mixed isomer LPC **3c** and it was 58.0 (HL-60) and 111.5 μM (MCF-7).

Results obtained in this study suggest that there is no strict relationship between the presence of the specific configuration (*cis*-9,*trans*-11 or *trans*-10,*cis*-12) of the conjugated bond system in the tested compounds and their cytotoxic activity. As reported in scientific studies, particular CLA isomers have different activities against various types of cancer cells.[6,12–15,39–51] The mechanism of action may be complex and could be attributed to alteration of lipid peroxidation, tissue fatty acid composition, eicosanoid metabolism, gene expression, cell-cycle regulation, cell proliferation, and apoptosis (reviewed by Kelley et al.).[52] It is possible that a particular isomer in the form of the free acid affects cell metabolism in a different way than in the form associated with a phospholipid. This may be related to hydrolysis and release of the acyl residue of only one of the CLA isomers from the phospholipid molecule owing to the regioselectivity and substrate specificity of cellular phospholipases or differences between the membrane transport of the compounds. These could explain why the more potent *trans*-10,*cis*-12 CLA isomer (HL-60, MCF-7) in the free form is less active than *cis*-9,*trans*-11 in the PC form (Table 3).

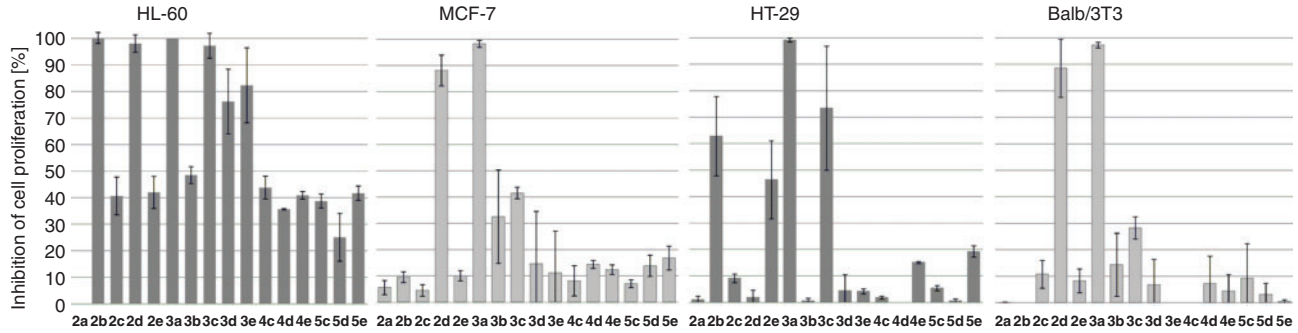


Fig. 2. Cell proliferation inhibition (%). An SRB or MTT assay was performed after 72-h exposure to concentrations of 100 μg mL^{-1} of the tested compounds. Values are means ± s.d., $n = 3$. Results that are significantly different from each other ($P < 0.05$): HL-60; **2a**, **b**; **2a**, **c**; **2a**, **d**; **2a**, **e**; **2b**, **c**; **2b**, **e**; **2c**, **d**; **2d**, **e**; **3a**, **b**; **3b**, **c**; **3b**, **e**; MCF-7; **2a**, **d**; **2b**, **c**; **2b**, **d**; **2c**, **d**; **2c**, **e**; **2d**, **e**; **3a**, **c**; **4d**, **5c**; HT-29: **2a**, **b**; **2a**, **c**; **2a**, **e**; **2b**, **c**; **2b**, **d**; **2c**, **e**; **2d**, **e**; **3a**, **b**; **3a**, **d**; **3a**, **e**; **3b**, **c**; **3c**, **d**; **3c**, **e**; **4c**, **e**; **4c**, **5e**; **4d**, **e**; **4d**, **5e**; **4e**, **5c**; **4e**, **5d**; **5c**, **d**; **5c**, **e**; **5e**, **d**; Balb/3T3; **1a**, **b**; **1a**, **c**; **1a**, **d**; **1a**, **e**; **1c**, **e**; **2a**, **d**; **2b**, **d**; **2c**, **d**; **2d**, **e**; **3a**, **b**; **3a**, **c**; **3a**, **d**; **3a**, **e**.

Table 3. The anti-proliferative in vitro activity of synthesized phosphatidylcholines on selected cell lines
SA, stearic acid; LA, linoleic acid; CLA, conjugated linoleic acid; mCLA, mixture of *c*9,*t*11 and *t*10,*c*12 conjugated linoleic acid isomers

Compounds	Acyl residue		Compounds' IC_{50}^{A} [μM]			
	sn-1	*sn*-2	HL-60	MCF-7	HT-29	Balb/3T3
2a	SA	SA	>126.6	>126.6	>126.6	>126.6
2b	LA	LA	31.2 ± 6.1	>127.9	64.6 ± 6.4	>127.9
2c	mCLA	mCLA	>127.9	>127.9	>127.9	>127.9
2d	*c*9,*t*11 CLA	*c*9,*t*11 CLA	29.4 ± 11.7	45.8 ± 6.6	>127.9	43.8 ± 9.5
2e	*t*10,*c*12 CLA	*t*10,*c*12 CLA	>127.9	>127.9	95.5 ± 39.9	>127.86
3a	SA	–	60.4 ± 2.0	59.8 ± 2.3	60.4 ± 0.1	59.1 ± 4.6
3b	LA	–	129.7 ± 47.7	>192.4	>192.4	>192.4
3c	mCLA	–	58.0 ± 2.3	>192.4	111.5 ± 56.5	>192.4
3d	*c*9,*t*11 CLA	–	96.1 ± 33.5	>192.4	>192.4	>192.4
3e	*t*10,*c*12 CLA	–	91.0 ± 73.0	>192.4	>192.4	>192.4
4c	SA	mCLA	>127.2	>127.2	>127.2	>127.2
4d	SA	*c*9,*t*11 CLA	>127.2	>127.2	>127.2	>127.2
4e	SA	*t*10,*c*12 CLA	>127.2	>127.2	>127.2	>127.2
5c	mCLA	SA	>127.2	>127.2	>127.2	>127.2
5d	*c*9,*t*11 CLA	SA	>127.2	>127.2	>127.2	>127.2
5e	*t*10,*c*12 CLA	SA	>127.2	>127.2	>127.2	>127.2

[A]The concentration of a compound that inhibits the proliferation of 50 % of the cancer cell population. Data are presented as mean ± s.d. of three independent experiments.

Conclusion

In summary, new *cis*-9,*trans*-11 and *trans*-10,*cis*-12 PC and lysophosphatidylcholine derivatives were synthesized in high yields and the in vitro cytotoxic activities of these compounds were investigated. Results obtained in this study suggest that there is no strict relationship between the presence of the specific configuration (*cis*-9,*trans*-11 or *trans*-10,*cis*-12) of the conjugated bond system in the tested compounds and their cytotoxic activity. However, the results confirmed that there are differences in activity between PC derivatives of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA. It seems that cytotoxic activity can be connected with the spatial structure of CLA residues in PC, which depends on the configuration of the double bonds. It is possible that the particular isomer in the form of the free acid affects cell metabolism in a different way than in the form associated with a phospholipid.

Experimental

General

All chemicals and reagents including stearic (**1a**) and linoleic (**1b**) acids were purchased from Sigma–Aldrich Chemical Co. The isomeric mixture of CLA (0.7 % LA; 48.5 % *cis*-9, *trans*-11; 48.3 % *trans*-10,*cis*-12; 2.5 % other CLA isomers, purity 97 %) (**1c**) and *trans*-10,*cis*-12 CLA (**1e**) (purity 96.5 %) was obtained according to the reported procedure.[11] *Cis*-9,*trans*-11 CLA (purity 94.2 %) (**1d**) was prepared according to the procedure described previously.[25] Phospholipase A_2 (Lecitase 10L) was a gift from Novozymes. The enantiomerically pure GPC was purchased from Bachem and converted to the cadmium chloride complex (GP**C** × $CdCl_2$) using the method described elsewhere.[21] 1,2-Di(conjugated) linoleoyl-*sn*-glycero-3-phosphocholine (**2c**) (purity 98 %) and 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (**2b**) were also prepared according to a procedure described previously.[21] All of the solvents used in liquid chromatography were of HPLC grade (Merck LiChrosolv Reagents) and were purchased from Merck.

TLC analyses were carried out on pre-coated silica gel 60 F_{254} plates (Merck). The compounds were detected using either a 0.05 % primuline solution (8 : 2 acetone/H_2O v/v) followed by UV (365 nm) visualization, or a solution of 10 g of $Ce(SO_4)_2$ and 20 g of phosphomolybdic acid in 1 L of 10 % H_2SO_4 followed by heating. Column chromatography was performed on silica gel (Kieselgel 60, 230–400 mesh (Merck)). The chemical structures of the synthesized compounds were confirmed by NMR spectroscopy and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). All of the ^1H, ^{13}C, and ^{31}P NMR spectra were recorded using a Bruker Avance DRX 300 spectrometer. Chemical shifts (^1H and ^{13}C) are given in ppm downfield from tetramethylsilane (TMS) as the internal standard. In ^{31}P NMR spectra, chemical shifts were referenced to 85 % H_3PO_4 as a standard. Coupling constant (J) values are given in hertz. HR-ESI mass spectra were obtained on a Bruker micrOTOF-Q.

Reaction progress and the purity of the obtained PC were monitored by HPLC. HPLC was performed on an Ultimate 3000 Dionex chromatograph equipped with a DGP-3600A dual-pump fluid control module, a TCC-3200 thermostatted column compartment, and a WPS-3000 autosampler. A Corona charged aerosol detector (CAD) from ESA Biosciences was used, with the following parameters: acquisition range: 100 pA, digital filter set to none, N_2 pressure: 0.24 MPa. The system and data acquisition were carried out using the *Chromeleon 6.80* software (Dionex Corporation). Analysis was carried out using a Betasil DIOL 5-μm column (Thermo, 150 × 2.1 mm). The injection volume was 15 mL in all of the experiments and the cooling temperature for the samples was 20°C. The column temperature was maintained at 30°C. The total time of analysis was 19 min. The mobile phase had a constant flow of 1.5 mL min^{-1}. Solvent A (1 % HCOOH, 0.1 % triethylamine (TEA) in water), solvent B (hexane), and solvent C (propan-2-ol) were used in gradient mode starting with 3 : 40 : 57 (A : B : C (vol-%/vol-%/vol-%)), at 4 min = 10 : 40 : 50, at 9 min = 10 : 40 : 50, at 9.1 min = 3 : 40 : 57 and at 19 min = 3 : 40 : 57.

The FA composition of the obtained PC was analysed by GC on an Agilent 6890N apparatus with a flame ionization detector (FID). The separation of FA methyl esters was achieved using a 70 % cyanopropylpolysilphenylene–siloxane column (TR FAME, 30 m × 0.25 mm × 0.25 μm). The initial oven temperature was 140°C held for 3 min, which was then increased to 190°C at a rate of 5°C min^{-1} and then to 260°C at a rate of 30°C min^{-1}. This was then held for 3 min while the injector temperature was 250°C. The FID temperature was set at 280°C. Hydrogen was used as a carrier gas.

Positional analysis of **4c**–**e** and **5c**–**e** was carried out according to the method described before.[21]

General Procedure for 1,2-Diacyl-sn-glycero-3-phosphocholines Synthesis

1,2-diacyl-*sn*-glycero-3-phosphocholines **2a**, **2d**, and **2e** were prepared according to the procedure described previously.[21]

1,2-Dioctadecanoyl-sn-glycero-3-phosphocholines ***2a***

White solid (864 mg, 96 %), purity (according to HPLC) 99 %, traces of stearoyl LPC. HPLC R_t 6.69 min. The chemical structure was confirmed by ^1H NMR and ^{13}C NMR. The spectral data were in accordance with those reported in the literature.[24]

1,2-Di-(9Z,11E-octadecadienoyl)-sn-glycero-3-phosphocholine ***2d***

The *title compound* was obtained as a colourless greasy solid (669 mg, 75 %), with 99 % purity (according to HPLC, R_t 7.01 min), traces of 9*Z*,11*E*-octadecadienoyl LPC. δ_H (300 MHz, $CDCl_3$) 6.28 (ddd, *J* 15.0, 11.0, 1.1, 2H, H-11d), 5.93 (t, *J* 11.0, 2H, H-10d), 5.64 (dt, *J* 15.0, 7.0, 2H, H-12d), 5.30 (dt, *J* 15.0, 7.0, 2H, H-9d), 5.18 (m, 1H, H-2), 4.38 (dd, *J* 12.0, 2.8, 1H, one of the H-1), 4.26–4.18 (m, 2H, H-α), 4.13 (dd, *J* 12.0, 7.4, 1H, one of the H-1), 3.92 (dd, *J* 12.7, 5.9, 2H, H-3), 3.84–3.74 (m, 2H, H-β), 3.34 (s, 9H, $N(CH_3)_3$), 2.34–2.21 (m, 4H, H-2d), 2.18–2.0 (m, 8H, H-8d, H-13d), 1.64–1.44 (m, 4H, H-3d), 1.42–1.11 (m, 32H, H-4d-7d, H-14d-17d), 0.88 (t, *J* 6.9, 6H, H-18d). δ_C (75 MHz, $CDCl_3$) 173.78 (C-1d in *sn*-2), 173.40 (C-1d in *sn*-1), 134.53 (C-12d), 129.55 (C-9d), 128.58 (C-10d), 125.40 (C-11d), 70.29 (C-2), 70.15 (C-2), 66.31 (C-β), 66.23 (C-β), 63.43 (C-3), 63.36 (C-3), 62.50 (C-1), 58.85 (C-α), 58.78 (C-α), 53.92 (–$N(CH_3)_3$), 34.04 (C-2d in *sn*-2), 33.82 (C-2d in *sn*-1), 32.61 (C-13d), 31.53 (C-16d), 29.64–28.59 (C-4d–7d, C-14d–15d), 27.43 (C-8d) 24.65 (C-3d in *sn*-2), 24.63 (C-3d in *sn*-1), 22.35 (C-17d), 13.68 (C-18d). Note: signals labelled 'd' are from the *cis*-9, *trans*-11 CLA moiety, those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) −0.42. *m/z* (HR-ESI) 782.5685; calc. for $C_{44}H_{80}NO_9P$: 782.5694 $[M + H]^+$.

*1,2-Di-(10E,12Z-octadecadienoyl)-*sn*-glycero-3-phosphocholine **2e***

The *title compound* was obtained as a colourless greasy solid (669 mg, 75 %) with 98 % purity (according to HPLC, R_t 7.03 min), traces of 10*E*,12*Z*-octadecadienoyl LPC. δ_H (300 MHz, $CDCl_3$) 6.29 (ddd, *J* 15.0, 10.9, 1.1, 2H, H-11e), 5.93 (t, *J* 10.9, 2H, H-12e), 5.64 (dt, *J* 15.0, 7.0, 2H, H-10e), 5.30 (dt, *J* 10.9, 7.4, 2H, H-13e), 5.19 (m, 1H, H-2), 4.39 (dd, *J* 12.0, 2.8, 1H, one of the H-1), 4.36–4.25 (m, 2H, H-α), 4.12 (dd, *J* 12.0, 7.4, 1H, one of the H-1), 3.93 (dd, *J* 12.7, 5.9, 2H, H-3), 3.84–3.74 (m, 2H, H-β), 3.36 (s, 9H, $N(CH_3)_3$), 2.34–2.22 (m, 4H, H-2e), 2.20–2.00 (m, 8H, H-9e and H-14b), 1.66–1.49 (m, 4H, H-3e), 1.44–1.21 (m, 32H, H-4e-8e and H-15e-17e), 0.88 (t, *J* 6.9, 6H, H-18e). δ_C (75 MHz, $CDCl_3$) 134.65 (C-10e), 130.28 (C-13e), 128.72 (C-12e), 125.82 (C-11e), 70.69 (C-2), 70.60 (C-2), 66.56 (C-β), 66.48 (C-β), 63.57 (C-3), 63.51 (C-3), 63.15 (C-1), 59.50 (C-α), 59.44 (C-α), 54.55 (–$N(CH_3)_3$), 34.45 (C-2e in *sn*-2), 34.26 (C-2e in *sn*-1), 33.04 (C-9e), 31.61 (C-16e), 29.76–29.08 (C-4e–8e and C-15e), 27.80 (C-14e) 25.10 (C-3e in *sn*-2), 25.02 (C-3e in *sn*-1), 22.69 (C-17e), 14.19 (C-18e). Note: signals labelled 'e' are from the *trans*-10,*cis*-12 CLA moiety, those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) −0.15. *m/z* (HR-ESI) 782.5676; calc. for $C_{44}H_{80}NO_9P$: 782.5694 $[M + H]^+$.

*General Procedure for 1,2-Diacyl-*sn*-glycero-3-phosphocholines Enzymatic Hydrolysis*

Hydrolysis of 1,2-diacyl-*sn*-glycero-3-phosphocholines **2a**–**e** was carried out according to the method described by Morgado et al.[30] Two solutions, A: 0.635 mmol of **2a**–**e** in 13.2 mL isooctane, and B: 174 mg (0.393 mmol) of sodium docusate (AOT) in 13.2 mL isooctane, were pre-incubated for ∼0.5 h at 40°C on a magnetic stirrer. Then, 262 µL of PLA_2 (10000 U mL^{-1}; 1 U is equivalent to the amount of enzyme producing 1 µmole of free fatty acid per minute at pH 8 and 40°C) and the same volume of Tris-HCl buffer (0.1 M, pH 8.5) with 0.75 M of $CaCl_2$ were then added to solution B and the reaction was started by adding solution A. The progress of the enzymatic reactions was monitored by TLC (65 : 25 : 4 $CHCl_3/CH_3OH/H_2O$ v/v/v). The reaction was carried out for 30 min. The enzyme was separated from the reaction mixture using diatomaceous earth (Celite® 545) and the solvent was evaporated to dryness at 40°C under vacuum. 1-Acyl LPCs **3a**–**e** were purified on a silica gel column (65 : 25 : 4 $CHCl_3/CH_3OH/H_2O$ v/v/v). Product-containing fractions of R_f 0.3 were collected and solvent evaporated under vacuum (45°C).

*1-Octadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine **3a***

The *title compound* was obtained as a white solid (329 mg, 99 %) with 99 % purity (according to HPLC, R_t 8.06 min), traces of 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine. δ_H (300 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) 4.07–3.98 (m, 2H, H-α), 3.96–3.83 (m, 2H, H-1), 3.80–3.58 (m, 3H, H-2 and H-3), 3.40–3.33 (m, 2H, H-β), 3.15 (s, 1H, -OH), 2.99 (s, 9H, $N(CH_3)_3$), 2.11 (t, *J* 7.6, 2H, H-2a), 1.44–1.31 (m, 2H, H-3a), 1.11–0.99 (m, 28H, H-4a–17a), 0.64 (t, *J* 6.6, 3H, H-18a). δ_C (75 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) 174.13 (C-1a in *sn*-1), 68.44 (C-2), 68.35 (C-2), 66.60 (C-β), 66.56 (C-β), 66.12 (C-3), 64.57 (C-1), 58.70 (C-α), 58.69 (C-α), 53.54, 53.69, 53.64 (–$N(CH_3)_3$), 33.74 (C-2a in *sn*-1), 31.59 (C-16a), 28.50–28.56 (C-7a–14a), 24.54 (C-3a), 22.33 (C-17a), 13.55 (C-18a). Note: signals labelled 'a' are from the stearoyl moiety, those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) −0.01. *m/z* (HR-ESI) 524.3728; calc. for $C_{26}H_{54}NO_7P$: 524.3711 $[M + H]^+$.

*1-(9Z,12Z)-Octadecadienoyl-2-hydroxy-*sn*-glycero-3-phosphocholine **3b***

The *title compound* was obtained as a colourless greasy solid (313 mg, 95 %) with 99 % purity (according to HPLC, R_t 8.28 min), traces of 1,2-di-(9*Z*,12*Z*-octadecadienoyl)-*sn*-glycero-3-phosphocholine. δ_H (300 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) 5.19–5.02 (m, 4H, H-9b, H-10b, H-12b, H13b), 4.07–3.97 (m, 2H, H-α), 3.95–3.82 (m, 2H, H-1), 3.80–3.58 (m, 3H, H-2 and H-3), 3.40–3.33 (m, 2H, H-β), 3.15 (s, 1H, -OH), 2.98 (s, 9H, $N(CH_3)_3$), 2.53 (t, *J* 5.9, 2H, H-11b), 2.11 (t, *J* 7.6, 2H, H-2b), 1.89–1.74 (m, 4H, H-8b, H-14b), 1.44–1.31 (m, 2H, H-3b), 1.19–1.00 (m, 28H, H-4b–7b, H-15b–17b), 0.65 (t, *J* 6.9, 3H, H-18b). δ_C (75 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) 174.06 (C-1b in *sn*-1), 129.79 (C-12), 129.61 (C-10), 127.70 (C-9), 127.56 (C-13), 68.43 (C-2), 68.34 (C-2), 66.60 (C-β), 66.52 (C-β), 66.10 (C-3), 64.57 (C-1), 58.75 (C-α), 58.69 (C-α), 53.72, 53.68, 53.63 (–$N(CH_3)_3$), 33.71 (C-2a in *sn*-1), 31.18 (C-16a), 29.26 (C-15b), 29.09–28.32 (C-4b–7b, C-14b), 26.82 (C-8), 25.24 (C-11b), 24.51 (C-3b), 22.20 (C-17b), 13.51 (C-18b). Note: signals labelled 'b' are from the linoleoyl moiety, those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) −0.71. *m/z* (HR-ESI) 518.3270; calc. for $C_{26}H_{50}NO_7P$: 518.3241 $[M + H]^+$.

*1-(Conjugated)linoleoyl-2-hydroxy-*sn*-glycero-3-phosphocholine **3c***

The *title compound* was obtained as a colourless greasy solid (294 mg, 89 %) with 99 % purity (according to HPLC, R_t 8.14 min), traces of 1,2-di-(conjugated)linoleoyl-*sn*-glycero-3-phosphocholine. δ_H (300 MHz, $CDCl_3$) 6.28 (m, 1H, H-11d or H-11e), 5.92 (m, 1H, H-10d or H-12e), 5.62 (m, 1H, H-12d or H-10e), 5.28 (m, 1H, H-9d or H-13e), 4.35–4.23 (m, 2H, H-α), 4.10–4.0 (m, 2H, H-1), 3.99–3.84 (m, 3H, H-2 and H-3), 3.82–3.67 (m, 2H, H-β), 3.47 (s, 1H, –OH), 3.30 (s, 9H, $N(CH_3)_3$), 2.30 (t, *J* 7.6, 2H, H-2d or H-2e), 2.17–1.97 (m, 4H, H-8d, H-13d or H-9e, H-14e), 1.64–1.47 (m, 2H, H-3d or H-3e), 1.41–1.19 (m, 16H, H-4d–7d, H-14d–17d or H-4e–8e, H-15e–17e), 0.88 (t, *J* 6.8, 3H, H-18d or H-18e). δ_C (75 MHz, $CDCl_3$) 174.12 (C-1d and C-1e in *sn*-1), 134.92 (C-12d), 134.64 (C-10e), 130.26 (C-13e), 129.92 (C-9d), 128.89 (C-10d), 128.76 (C-12e), 125.84 (C-11e), 125.73 (C-11d), 68.84 (C-2), 68.74 (C-2), 67.29 (C-β), 67.23 (C-β), 66.37 (C-3), 66.29 (C-3), 65.68 (C-1), 59.65 (C-α), 54.48 (–$N(CH_3)_3$), 34.28 (C-2d and C-2e in *sn*-1), 33.07 (C-13d and C-9e), 31.90 (C-16d), 31.63 (C-16e), 30.02–29.22 (C-4d–7d, C-14d–15d, C-4e–8e, and C-15e), 27.88 (C-8d), 27.82 (C-14e), 25.08 (C-3d and C-3e in *sn*-1), 22.77 (C-17d), 22.71 (C-17e), 14.26 (C-18d), 14.22 (C-18e). Note: signals labelled 'd' are from the *cis*-9,*trans*-11 CLA moiety, those labelled 'e' are from *trans*-10,*cis*-12 CLA moiety, those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) −0.61. *m/z* (HR-ESI) 518.3265; calc. for $C_{26}H_{50}NO_7P$: 518.3241 $[M + H]^+$.

*1-(9Z,11E)-Octadecadienoyl-2-hydroxy-*sn*-glycero-3-phosphocholine **3d***

The *title compound* was obtained as a colourless greasy solid (310 mg, 94 %) with 99 % purity (according to HPLC,

R_t 8.15 min), traces of 1,2-di-(9*Z*,11*Z*-octadecadienoyl)-*sn*-glycero-3-phosphocholine. δ_H (300 MHz, $CDCl_3$) 6.28 (ddd, *J* 15.0, 11.0, 1.1, 1H, H-11d), 5.93 (t, *J* 11.0, 1H, H-10d), 5.64 (dt, *J* 15.0, 7.0, 1H, H-12d), 5.28 (dt, *J* 11.0, 7.5, 1H, H-9d), 4.37–4.23 (m, 2H, H-α), 4.12–4.03 (m, 2H, H-1), 3.99–3.86 (m, 3H, H-2 and H-3), 3.79–3.70 (m, 2H, H-β), 3.47 (m, 1H, –OH), 3.30 (s, 9H, $N(CH_3)_3$), 2.31 (t, *J* 7.6, 2H, H-2d), 2.19–1.97 (m, 4H, H-8d, H-13d), 1.64–1.47 (m, 2H, H-3d), 1.41–1.17 (m, 16H, H-4d–7d, H-14d–17d), 0.88 (t, *J* 6.9, 3H, H-18d). δ_C (75 MHz, $CDCl_3$) 174.10 (C-1d in *sn*-1), 134.93 (C-12d), 129.93 (C-9d), 128.83 (C-10d), 125.73 (C-11d), 68.84 (C-2), 68.76 (C-2), 67.29 (C-β), 67.25 (C-β), 66.38 (C-3), 66.29 (C-3), 65.39 (C-1), 59.64 (C-α), 54.48 (–$N(CH_3)_3$), 34.28 (C-2d in *sn*-1), 33.07 (C-13d), 31.90 (C-16d), 30.11–29.24 (C-4d–7d, C-14d–15d), 27.88 (C-8d), 25.08 (C-3d in *sn*-1), 22.77 (C-17d), 14.26 (C-18d). Note: signals labelled 'd' are from the *cis*-9,*trans*-11 CLA moiety; those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) −0.37. *m/z* (HR-ESI) 518.3232; calc. for $C_{26}H_{50}NO_7P$: 518.3241 $[M + H]^+$.

*1-(10*E*,12Z)-Octadecadienoyl-2-hydroxy*-sn-*glycero-3-phosphocholine* ***3e***

The *title compound* was obtained as a colourless greasy solid (307 mg, 93 %) with 99 % purity (according to HPLC, R_t 8.16 min), traces of 1,2-di-(10*E*,12*Z*-octadecadienoyl)-*sn*-glycero-3-phosphocholine. δ_H (300 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) 6.03 (ddd, J 15.0, 10.9, 1.1, 1H, H-11e), 5.67 (t, *J* 10.9, 1H, H-12e), 5.38 (dt, *J* 15.0 7.0, 1H, H-10e), 5.03 (dt, *J* 10.9, 7.4, 1H, H-13e), 4.05–3.96 (m, 2H, H-α), 3.94–3.81 (m, 2H, H-1), 3.78–3.56 (m, 3H, H-2 and H-3), 3.38–3.31 (m, 2H, H-β), 3.11 (m, 1H, –OH), 2.96 (s, 9H, $N(CH_3)_3$), 2.09 (t, *J* 7.6, 2H, H-2e), 2.95–1.75 (m, 4H, H-9e and H-14e), 1.42–1.29 (m, 2H, H-3e), 1.19–0.97 (m, 16H, H-4e–8e and H-15e–17e), 0.63 (t, *J* 6.9, 3H, H-18e). δ_C (75 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) 174.34 (C-1e in *sn*-1), 134.37 (C-10e), 129.87 (C-13e), 128.57 (C-12e), 125.64 (C-11e), 68.57 (C-2), 68.58 (C-2), 66.83 (C-β), 66.76 (C-β), 66.34 (C-3), 64.94 (C-1), 59.02 (C-α), 58.96 (C-α), 53.94, 53.89, 53.85 (–$N(CH_3)_3$), 33.95 (C-2e in *sn*-1), 33.75 (C-9e), 31.36 (C-16e), 29.54–28.79 (C-4e-8e and C-15e), 27.50 (C-14e), 24.76 (C-3e in *sn*-1), 22.43 (C-17e), 13.71 (C-18e). Note: signals labelled 'e' are from the *trans*-10,*cis*-12 CLA moiety, those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, 2 : 1 $CDCl_3/CD_3OD$ v/v) 0.69. *m/z* (HR-ESI) 518.3261; calc. for $C_{26}H_{50}NO_7P$: 518.3241 $[M + H]^+$.

*General Procedure for 1-Octadecanoyl-2-acyl-*sn-*glycero-3-phosphocholine Synthesis*

Compounds **4c**–**e** were prepared from 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (**3a**) (100 mg, 0.191 mmol) by adding 106 mg (0.381 mmol) of a mixture of CLA isomers (**1c**), *cis*-9,*trans*-11 (**1d**) or *trans*-10,*cis*-12 CLA (**1e**) dissolved in 5.7 mL of anhydrous CH_2Cl_2, DMAP (53.8 mg, 0.191 mmol), and finally a solution of DCC (119 mg, 0.419 mmol) in 1.9 mL of CH_2Cl_2. The reaction was carried out for 48 h under a nitrogen atmosphere in the dark and at ambient temperature. The precipitate that was formed in the reaction mixture was removed using a Shott funnel. Ion-exchange resin (Dowex 50W X8, H^+ form) was added to the filtrate and the mixture was stirred for 30 min to dislodge DMAP. The Dowex resin was filtered off and the solvent was evaporated under vacuum. The crude PC was purified on a silica gel column (65 : 25 : 4 $CHCl_3/CH_3OH/H_2O$ v/v/v). Product-containing fractions of R_f 0.4 were collected and solvent evaporated under vacuum (45°C).

*1-Octadecanoyl-2-(conjugated)linoleoyl-*sn-*glycero-3-phosphocholine* ***4c***

The *title compound* was obtained as a colourless greasy solid (119 mg, 79 %) with 98 % purity (according to HPLC, R_t 6.79 min), traces of 1-octadecanoyl LPC. δ_H (300 MHz, $CDCl_3$) 6.29 (m, 1H, H-11d or H-11e), 5.93 (m, 1H, H-10d or H-12e), 5.64 (m, 1H, H-12d or H-10e), 5.30 (m, 1H, H-9d or H-13e), 5.19 (m, 1H, H-2), 4.43–4.21 (m, 2H, H-α), 4.39 (dd, *J* 12.3, 2.9, 1H, one of the H-1), 4.43–4.21 (m, 2H, H-α), 4.12 (dd, *J* 12.0, 7.2, 1H, one of the H-1), 4.00–3.85 (m, 2H, H-3), 3.84–3.73 (m, 2H, H-β), 3.36 (s, 9H, $N(CH_3)_3$), 2.35–2.22 (m, 4H, H-2d or H-2e and H-2a), 2.19–1.98 (m, 4H, H-8d and H-13d or H-9e and H-14e), 1.65–1.48 (m, 4H, H-3d or H-3e and H-3a), 1.40–1.13 (m, 44H, H-4d–7d, H-14d–17d or H-4e–8e, H-15e–17e, and H-4a–17a), 0.93–0.81 (t, *J* 6.7, 6H, H-18a and H-18d or H-18e). δ_C (75 MHz, $CDCl_3$) 173.73 (C-1d or C-1e in *sn*-2), 173.35 (C-1a in *sn*-1), 134.95(C-12d), 134.67 (C-10e), 130.30 (C-13e), 129.91 (C-9d), 128.86 (C-10d), 128.72 (C-12e), 125.83 (C-11e), 125.71 (C-11d), 70.74 (C-2), 70.64 (C-2), 66.52 (C-β), 66.49 (C-β), 63.63 (C-3), 63.56 (C-3), 63.15 (C-1), 59.53 (C-α), 59.47 (C-α), 54.66 (–$N(CH_3)_3$), 34.47 (C-2d and C-2e in *sn*-2), 34.29 (C-2a in *sn*-1), 32.54 (C-13d and C-9e), 32.08 (C-16a), 31.90 (C-16d), 31.63 (C-16e), 30.14–29.09 (C-4a–15a, C-4d–7d, C-14d–15d, C-4e–8e, C-15e), 27.82 (C-8d and C-14e), 25.11 (C-3a), 25.05 (C-3d or C-3e), 22.84 (C-17a), 22.77 (C-17d), 22.71 (C-17e), 14.27 (C-16a and C-18d or C-18e). Note: signals labelled 'd' are from the *cis*-9,*trans*-11 CLA moiety, those labelled 'e' are from *trans*-10,*cis*-12 CLA moiety, those labelled 'a' are from stearoyl moiety and those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) 16.48. *m/z* (HR-ESI) 784.5874; calc. for $C_{44}H_{84}NO_8P$: 784.5851 $[M + H]^+$.

*1-Octadecanoyl-2-(9*Z*,11*E*)-octadecadienoyl-*sn-*glycero-3-phosphocholine* ***4d***

The *title compound* was obtained as a colourless greasy solid (113 mg, 75 %) with 98 % purity (according to HPLC, R_t 6.74 min), traces of 1-octadecanoyl LPC. δ_H (300 MHz, $CDCl_3$) 6.27 (ddd, *J* 15.0, 11.0, 1.1, 1H, H-11d), 5.93 (t, *J* 11.0, 1H, H-10d), 5.64 (dt, *J* 15.0, 7.0, 1H, H-12d), 5.29 (dt, *J* 15.0, 7.0, 1H, H-9d), 5.18 (m, 1H, H-2), 4.38 (dd, 12.0, 2.8, 1H, one of the H-1), 4.34–4.22 (m, 2H, H-α), 4.11 (dd, *J* 12.0, 7.4, 1H, one of the H-1), 3.97–3.85 (m, 2H, H-3), 3.83–3.73 (m, 2H, H-β), 3.34 (s, 9H, $N(CH_3)_3$), 2.34–2.23 (m, 4H, H-2d and H-2a), 2.20–2.03 (m, 4H, H-8d and H-13d), 1.65–1.48 (m, 4H, H-3d and H-3a), 1.43–1.18 (m, 44H, H-4d–7d, H-14d–17d, and H-4a–17a), 0.92–0.81 (m, 6H, H-18a and H-18d). δ_C (75 MHz, $CDCl_3$) 173.72 (C-1d in *sn*-2), 173.34 (C-1a in *sn*-1), 134.93 (C-12d), 129.94 (C-9d), 128.86 (C-10d), 128.70 (C-11d), 70.69 (C-2), 70.59 (C-2), 66.55 (C-β), 66.48 (C-β), 63.61 (C-3), 63.54 (C-3), 63.14 (C-1), 59.51 (C-α), 59.46 (C-α), 54.56 (–$N(CH_3)_3$), 34.45 (C-2d in *sn*-2), 34.28 (C-2a in *sn*-1), 33.05 (C-13d), 32.07 (C-16a), 31.89 (C-16d), 30.17–28.87 (C-4a–15a, C-4d–7d, C-14d–15d), 27.84 (C-8d), 25.10 (C-3a), 25.05 (C-3d), 22.83 (C-17a), 22.76 (C-17d),14.27 (C-16a and C-18d). Note: signals labelled 'd' are from the *cis*-9,*trans*-11 CLA moiety, those labelled 'a' are from the stearoyl moiety and those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) 16.48. *m/z* (HR-ESI) 784.5830; calc. for $C_{44}H_{84}NO_8P$: 784.5851 $[M + H]^+$.

1-Octadecanoyl-2-(10E,12Z)-octadecadienoyl-sn-*glycero-3-phosphocholine* ***4e***

The *title compound* was obtained as a colourless greasy solid (110 mg, 73 %) with 99 % purity (according to HPLC, R_t 6.72 min), traces of 1-octadecanoyl LPC. δ_H (300 MHz, $CDCl_3$) 6.28 (ddd, *J* 15.0, 10.9, 1.1, 1H, H-11e), 5.93 (t, *J* 10.9, 1H, H-12e), 5.64 (dt, *J* 15.0 7.0, 1H, H-10e), 5.29 (dt, *J* 10.9, 7.4, 1H, H-13e), 5.19 (m, 1H, H-2), 4.39 (dd, *J* 12.0, 2.8, 1H, one of the H-1), 4.34–4.25 (m, 2H, H-α), 4.12 (dd, *J* 12.0, 7.4, 1H, one of the H-1), 3.98–3.86 (m, 2H, H-3), 3.83–3.75 (m, 2H, H-β), 3.35 (s, 9H, $N(CH_3)_3$), 2.35–2.22 (m, 4H, H-2e and H-2a), 2.20–2.03 (m, 4H, H-9e and H-14e), 1.65–1.48 (m, 4H, H-3e and H-3a), 1.46–1.18 (m, 44H, H-4e–8e, H-15e–17e, and H-4a–17a), 0.97–0.81 (m, 6H, H-18a and H-18e). δ_C (75 MHz, $CDCl_3$) 173.73 (C-1e in *sn*-2), 173.35 (C-1a in *sn*-1), 134.68 (C-10e), 130.31 (C-13e), 128.73 (C-12e), 125.84 (C-11e), 70.77 (C-2), 70.67 (C-2), 66.46 (C-β), 66.50 (C-β), 63.64 (C-3), 63.58 (C-3), 63.16 (C-1), 59.54 (C-α), 59.48 (C-α), 54.56 (–$N(CH_3)_3$), 34.46 (C-2e in *sn*-2), 34.29 (C-2a in *sn*-1), 32.55 (C-9e), 32.09 (C-16a), 31.63 (C-16e), 30.15–28.97 (C-4a–15a, C-4e–8e, C-15e), 27.83 (C-14e), 25.11 (C-3a), 25.05 (C-3e), 22.84 (C-17a), 22.71 (C-17e),14.27 (C-16a and C-18e). Note: signals labelled 'e' are from the *trans*-10,*cis*-12 CLA moiety, those labelled 'a' are from the stearoyl moiety and those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) −0.20. *m/z* (HR-ESI) 784.5829; calc. for $C_{44}H_{84}NO_8P$: 784.5851 $[M + H]^+$.

General Procedure for 1-Acyl-2-octadecanoyl-sn-*glycero-3-phosphocholine Synthesis*

Compounds **5c**, **5d**, **5e** were prepared from **3c**, **3d**, **3e** (100 mg 0.192 mmol) respectively, by adding 109 mg (0.384 mmol) of stearic acid (**1a**) dissolved in 5.8 mL of anhydrous CH_2Cl_2, DMAP (54.3 mg, 0.192 mmol), and finally a solution of DCC (120 mg, 0.423 mmol) in 1.9 mL of CH_2Cl_2. The reaction was carried out for 48 h under a nitrogen atmosphere in the dark and at ambient temperature. The product was extracted according to the method described for compounds **4c**–**d**.

1-(Conjugated)linoleoyl-2-octadecanoyl-sn-*glycero-3-phosphocholine* ***5c***

The *title compound* was obtained as a colourless greasy solid (121 mg, 80 %) with 99 % purity (according to HPLC, R_t 6.73 min), traces of 1-(conjugated)lineoyl LPC. δ_H (300 MHz, $CDCl_3$) 6.28 (m, 1H, H-11d and H-11e), 5.95 (m, 1H, H-10d and H-12e), 5.65 (m, 1H, H-12d and H-10e), 5.31 (m, 1H, H-9d and H-13e), 5.19 (m, 1H, H-2), 4.39 (dd, *J* 12.3, 2.9, 1H, one of the H-1), 4.37–4.26 (m, 2H, H-α), 4.11 (dd, *J* 12.0, 7.2, 1H, one of the H-1), 4.00–3.86 (m, 2H, H-3), 3.84–3.75 (m, 2H, H-β), 3.36 (s, 9H, $N(CH_3)_3$), 2.35–2.22 (m, 4H, H-2d or H-2e and H-2a), 2.21–1.99 (m, 4H, H-8d and H-13d or H-9e and H-14e), 1.65–1.48 (m, 4H, H-3d or H-3e and H-3a), 1.44–1.18 (m, 44H, H-4d–7d, H-14d–17d or H-4e–8e, H-15e–17e and H-4a–17a), 0.94–0.82 (m, 6H, H-18a and H-18d or H-18e). δ_C (75 MHz, $CDCl_3$) 173.69 (C-1a in *sn*-2), 173.37 (C-1d or C-1e in *sn*-1), 134.93 (C-12d), 134.67 (C-10e), 130.28 (C-13e), 129.96 (C-9d), 128.85 (C-10d), 128.71 (C-12e), 125.81 (C-11e), 125.70 (C-11d), 70.69 (C-2), 70.60 (C-2), 66.58 (C-β), 66.52 (C-β), 63.59 (C-3), 63.54 (C-3), 63.15 (C-1), 59.48 (C-α), 59.43 (C-α), 54.59 (–$N(CH_3)_3$), 34.48 (C-2a in *sn*-2), 34.25 (C-2d and C-2e in *sn*-1), 33.05 (C-13d and C-9e), 32.07 (C-16a), 31.89 (C-16d), 31.62 (C-16e), 30.11–28.95 (C-4a–15a, C-4d–7d, C-14d–15d, C-4e–8e, C-15e), 27.81 (C-8d and C-14e), 25.13 (C-3a), 25.02 (C-3d or C-3e), 22.83 (C-17a), 22.76 (C-17d), 22.70 (C-17e),14.25 (C-16a and C-18d or C-18e). Note: signals labelled 'd' are from the *cis*-9,*trans*-11 CLA moiety, those labelled 'e' are from the *trans*-10,*cis*-12 CLA moiety, those labelled 'a' are from the stearoyl moiety and those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) −0.17. *m/z* (HR-ESI) 784.5859; calc. for $C_{44}H_{84}NO_8P$: 784.5851 $[M + H]^+$.

1-(9Z,11E)-Octadecadienoyl-2-octadecanoyl-sn-*glycero-3-phosphocholine* ***5d***

The *title compound* was obtained as a colourless greasy solid (121 mg, 80 %) with 98 % purity (according to HPLC, R_t 6.74 min), traces of 1-(9*Z*,11*E*)-octadecadienoyl LPC. δ_H (300 MHz, $CDCl_3$) 6.27 (ddd, *J* 15.0, 11.0, 1.1, 1H, H-11d), 5.93 (t, *J* 11.0, 1H, H-10d), 5.64 (dt, *J* 15.0, 7.0, 1H, H-12d), 5.29 (dt, *J* 15.0, 7.0, 1H, H-9d), 5.18 (m, 1H, H-2), 4.39 (dd, 12.0, 2.8, 1H, one of the H-1), 4.36–4.26 (m, 2H, H-α), 4.12 (dd, *J* 12.0, 7.4, 1H, one of the H-1), 3.97–3.86 (m, 2H, H-3), 3.82–3.73 (m, 2H, H-β), 3.36 (s, 9H, $N(CH_3)_3$), 2.35–2.21 (m, 4H, H-2d and H-2a), 2.19–2.03 (m, 4H, H-8d and H-13d), 1.65–1.48 (m, 4H, H-3d and H-3a), 1.43–1.18 (m, 44H, H-4d–7d, H-14d–17d, and H-4a–17a), 0.92–0.81 (m, 6H, H-18a and H-18d). δ_C (75 MHz, $CDCl_3$) 173.69 (C-1a in *sn*-2), 173.38 (C-1d in *sn*-1), 134.94 (C-12d), 129.97 (C-9d), 128.84 (C-10d), 125.69 (C-11d), 70.67 (C-2), 70.58 (C-2), 66.57 (C-β), 66.54 (C-β), 63.60 (C-3), 63.58 (C-3), 63.14 (C-1), 59.50 (C-α), 59.46 (C-α), 54.60 (−$N(CH_3)_3$), 34.47 (C-2a in *sn*-2), 34.25 (C-2d in *sn*-1), 33.05 (C-13d), 32.07 (C-16a), 31.89 (C-16d), 30.20–28.93 (C-4a–15a, C-4d–7d, C-14d–15d), 27.83 (C-8d), 25.13 (C-3a), 25.01 (C-3d), 22.84 (C-17a), 22.76 (C-17d), 14.26 (C-16a and C-18d). Note: signals labelled 'd' are from the *cis*-9,*trans*-11 CLA moiety, those labelled 'a' are from the stearoyl moiety and those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) −0.21. *m/z* (HR-ESI) 784.5830; calc. for $C_{44}H_{84}NO_8P$: 784.5851 $[M + H]^+$.

1-(10E,12Z)-Octadecadienoyl-2-octadecanoyl-sn-*glycero-3-phosphocholine* ***5e***

The *title compound* was obtained as a colourless greasy solid (124 mg, 82 %) with 99 % purity (according to HPLC, R_t 6.73 min), traces of 1-(10*E*,12*Z*)-octadecadienoyl LPC. δ_H (300 MHz, $CDCl_3$) 6.28 (ddd, *J* 15.0, 10.9, 1.1, 1H, H-11e), 5.93 (t, *J* 10.9, 1H, H-12e), 5.64 (dt, *J* 15.0 7.0, 1H, H-10e), 5.29 (dt, *J* 10.9, 7.4, 1H, H-13e), 5.19 (m, 1H, H-2), 4.39 (dd, *J* 12.0, 2.8, 1H, one of the H-1), 4.36–4.23 (m, 2H, H-α), 4.11 (dd, *J* 12.0, 7.4, 1H, one of the H-1), 4.00–3.86 (m, 2H, H-3), 3.85–3.73 (m, 2H, H-β), 3.35 (s, 9H, $N(CH_3)_3$), 2.35–2.22 (m, 4H, H-2e and H-2a), 2.20–2.02 (m, 4H, H-9e and H-14e), 1.65–1.48 (m, 4H, H-3e and H-3a), 1.46–1.18 (m, 44H, H-4e–8e, H-15e–17e, and H-4a–17a), 0.97–0.81 (m, 6H, H-18a and H-18e). δ_C (75 MHz, $CDCl_3$) 173.69 (C-1a in *sn*-2), 173.36 (C-1e in *sn*-1), 134.67 (C-10e), 130.28 (C-13e), 128.72 (C-12e), 125.81 (C-11e), 70.69 (C-2), 70.60 (C-2), 66.56 (C-β), 66.52 (C-β), 63.59 (C-3), 63.55 (C-3), 63.14 (C-1), 59.48 (C-α), 59.43 (C-α), 54.60 (–$N(CH_3)_3$), 34.48 (C-2a in *sn*-2), 34.26 (C-2e in *sn*-1), 33.04 (C-9e), 32.07 (C-16a), 31.62 (C-16e), 30.09–28.87 (C-4a–15a, C-4e–8e, C-15e), 27.81 (C-14e), 25.13 (C-3a), 25.03 (C-3e), 22.83 (C-17a), 22.70 (C-17e),14.25 (C-16a and C-18e). Note: signals labelled 'e' are from the *trans*-10,*cis*-12

CLA moiety, those labelled 'a' are from the stearoyl moiety and those labelled 'α,β' are from the choline moiety. δ_P (121 MHz, $CDCl_3$) −0.24. *m/z* (HR-ESI) 784.5877; calc. for $C_{44}H_{84}NO_8P$: 784.5851 $[M+H]^+$.

Cytotoxicity Bioassays

Breast cancer (MCF-7), human promyelocytic leukaemia (HL-60), colon cancer (HT-29) and mouse fibroblast (Balb/3T3) cell lines were obtained from the American Type Culture Collection (Rockville, MD). All the cell lines were maintained at the Institute of Immunology and Experimental Therapy, Wrocław, Poland. Propagation of all cell lines and the in vitro cytotoxic tests for all compounds that were examined using the sulforhodamine B (SRB) assay for adherent cells (MCF-7, HT-29 and Balb/3T3) and methylthiazoltetrazolium (MTT) assay for non-adherent cells (HL-60) were carried out according to the method described before.[21]

Results Evaluation

IC_{50} values were calculated for each experiment separately and mean values ± s.d. are presented in Tables 2 and 3. Each compound was tested at every concentration in triplicate in a single experiment, which was repeated three times.

Supplementary Material

^1H and ^{13}C NMR spectra for compounds **2d**, **2e**, **3a**–**e**, **4c**–**e**, and **5c**–**e** are available on the Journal's website.

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References

[1] G. V. Halade, M. M. Rahman, P. J. Williams, G. J. Fernandes, *J. Nutr. Biochem.* **2011**, *22*, 459. doi:10.1016/J.JNUTBIO.2010.03.015
[2] Y. Park, K. J. Albright, W. Liu, J. M. Storkson, M. E. Cook, M. W. Pariza, *Lipids* **1997**, *32*, 853. doi:10.1007/S11745-997-0109-X
[3] S. E. Steck, A. M. Chalecki, P. Miller, J. Conway, G. L. Austin, J. W. Hardin, C. D. Albright, P. Thuillier, *J. Nutr.* **2007**, *137*, 1188.
[4] L. D. Whigham, A. C. Watras, D. A. Schoeller, *Am. J. Clin. Nutr.* **2007**, *85*, 1203.
[5] M. Futakuchi, J. L. Cheng, M. Hirose, N. Kimoto, Y.-M. Cho, T. Iwata, M. Kasai, S. Tokudome, T. Shirai, *Cancer Lett.* **2002**, *178*, 131. doi:10.1016/S0304-3835(01)00860-6
[6] M. Q. Kemp, B. D. Jeffy, D. F. Romagnolo, *J. Nutr.* **2003**, *133*, 3670.
[7] X.-M. Tao, J.-C. Wang, J.-B. Wang, Q. Feng, S.-Y. Gao, L.-R. Zhang, Q. Zhang, *Eur. J. Pharm. Biopharm.* **2012**, *82*, 401. doi:10.1016/J.EJPB.2012.06.007
[8] W. K. Bae, J. H. Lee, S. J. Lee, M. S. Park, J. E. Hwang, H. J. Shim, S. H. Cho, D.-D. Guo, C.-S. Cho, I.-K. Park, *J. Nanosci. Nanotechnol.* **2011**, *11*, 1425. doi:10.1166/JNN.2011.3403
[9] Y. Park, M. Juárez, M. Ramos, G. Haenlein, *Small Rumin. Res.* **2007**, *68*, 88. doi:10.1016/J.SMALLRUMRES.2006.09.013
[10] S. Fritsche, J. Fritsche, *J. Am. Oil Chem. Soc.* **1998**, *75*, 1449. doi:10.1007/S11746-998-0199-0
[11] N. Niezgoda, P. Mituła, C. Wawrzeńczyk, *Przem. Chem.* **2011**, *90*, 949.
[12] P. Tanmahasamut, J. Liu, L. B. Hendry, N. Sidell, *J. Nutr.* **2004**, *134*, 674.
[13] F. Beppu, M. Hosokawa, L. Tanaka, H. Kohno, T. Tanaka, K. Miyashita, *J. Nutr. Biochem.* **2006**, *17*, 830. doi:10.1016/J.JNUTBIO.2006.01.007
[14] M. Yamasaki, H. Chujo, Y. Koga, A. Oishi, T. Rikimaru, M. Shimada, K. Sugimachi, H. Tachibana, K. Yamada, *Cancer Lett.* **2002**, *188*, 171. doi:10.1016/S0304-3835(02)00459-7
[15] J. D. Palombo, A. Ganguly, B. R. Bistrian, M. P. Menard, *Cancer Lett.* **2002**, *177*, 163. doi:10.1016/S0304-3835(01)00796-0
[16] A. El Roz, J. Bard, J. Huvelin, H. Nazih, *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2013**, *88*, 265. doi:10.1016/J.PLEFA.2012.12.006
[17] Y. Kim, R. Cerbo, C. Hah, K. Bahn, J. Kim, Y. Ha, *J. Food Sci.* **2008**, *73*, T7. doi:10.1111/J.1750-3841.2007.00584.X
[18] A.-M. Florea, D. Büsselberg, *Cancers* **2011**, *3*, 1351. doi:10.3390/CANCERS3011351
[19] L. Brannon-Peppas, J. O. Blanchette, *Adv. Drug Deliv. Rev.* **2012**, *64*, 206. doi:10.1016/J.ADDR.2012.09.033
[20] V. P. Carnielli, G. Verlato, F. Pederzini, I. Luijendijk, A. Boerlage, D. Pedrotti, P. Sauer, *Am. J. Clin. Nutr.* **1998**, *67*, 97.
[21] N. Niezgoda, P. Mituła, K. Kempińska, J. Wietrzyk, C. Wawrzeńczyk, *Aust. J. Chem.* **2013**, *66*, 354.
[22] C. M. Gupta, R. Radhakrishnan, H. G. P. Khorana, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 4315. doi:10.1073/PNAS.74.10.4315
[23] A. Singh, *J. Lipid Res.* **1990**, *31*, 1522.
[24] S. Ali, R. Bittman, *J. Org. Chem.* **1988**, *53*, 5547. doi:10.1021/JO00258A031
[25] N. Niezgoda, C. Wawrzeńczyk, *J. Mol. Catal., B: Enzym.* **2014**, *100*, 40. doi:10.1016/J.MOLCATB.2013.11.019
[26] P. D'Arrigo, S. Servi, *Molecules* **2010**, *15*, 1354. doi:10.3390/MOLECULES15031354
[27] E. Fasoli, A. Arnone, A. Caligiuri, P. D'Arrigo, L. de Ferra, S. Servi, *Org. Biomol. Chem.* **2006**, *4*, 2974. doi:10.1039/B604636C
[28] P. D'Arrigo, E. Fasoli, G. Pedrocchi-Fantoni, C. Rossi, C. Saraceno, D. Tessaro, S. Servi, *Chem. Phys. Lipids* **2007**, *147*, 113. doi:10.1016/J.CHEMPHYSLIP.2007.03.008
[29] F. H. Mattson, R. A. Volpenhein, *J. Lipid Res.* **1962**, *3*, 281.
[30] M. Morgado, J. Cabral, D. Prazeres, *J. Chem. Technol. Biotechnol.* **1995**, *63*, 181. doi:10.1002/JCTB.280630214
[31] D. Adlercreutz, E. Wehtje, *J. Am. Oil Chem. Soc.* **2004**, *81*, 553. doi:10.1007/S11746-006-0940-5
[32] D. Adlercreutz, H. Budde, E. Wehtje, *Biotechnol. Bioeng.* **2002**, *78*, 403. doi:10.1002/BIT.10225
[33] A. Chojnacka, W. Gładkowski, G. Kiełbowicz, C. Wawrzeńczyk, *Biotechnol. Lett.* **2009**, *31*, 705. doi:10.1007/S10529-009-9915-6
[34] D. Adlercreutz, E. Wehtje, *J. Am. Oil Chem. Soc.* **2001**, *78*, 1007. doi:10.1007/S11746-001-0379-8
[35] G. Kiełbowicz, D. Smuga, W. Gładkowski, A. Chojnacka, C. Wawrzeńczyk, *Talanta* **2012**, *94*, 22. doi:10.1016/J.TALANTA.2012.01.018
[36] A. Plueckthun, E. A. Dennis, *Biochemistry* **1982**, *21*, 1743. doi:10.1021/BI00537A007
[37] C. Degen, N. Habermann, S. Piegholdt, M. Glei, G. Jahreis, *Toxicol. In Vitro* **2012**, *26*, 985. doi:10.1016/J.TIV.2012.05.002
[38] M. Puszyńska-Tuszkanow, T. Grabowski, M. Daszkiewicz, J. Wietrzyk, B. Filip, G. Maciejewska, M. Cieślak-Golonka, *J. Inorg. Biochem.* **2011**, *105*, 17. doi:10.1016/J.JINORGBIO.2010.09.013
[39] N. E. Hubbard, D. Lim, K. L. Erickson, *Cancer Lett.* **2003**, *190*, 13. doi:10.1016/S0304-3835(02)00515-3
[40] J. Rajakangas, S. Basu, I. Salminen, M. Mutanen, *J. Nutr.* **2003**, *133*, 1943.
[41] M. M. Ip, S. O. McGee, P. A. Masso-Welch, C. Ip, X. Meng, L. Ou, S. Shoemaker, *Carcinogenesis* **2007**, *28*, 1269. doi:10.1093/CARCIN/BGM018
[42] H. Chujo, M. Yamasaki, S. Nou, N. Koyanagi, H. Tachibana, K. Yamada, *Cancer Lett.* **2003**, *202*, 81. doi:10.1016/S0304-3835(03)00478-6
[43] A. De la Torre, E. Debiton, D. Durand, J. M. Chardigny, O. Berdeaux, O. Loreau, C. Barthomeuf, D. Bauchart, D. Gruffat, *Anticancer Res.* **2005**, *25*, 3943.
[44] J. R. Liu, B. Q. Chen, Y. M. Yang, X. L. Wang, Y. B. Xue, Y. M. Zheng, R. H. Liu, *World J. Gastroenterol.* **2002**, *8*, 999.
[45] E. J. Kim, H. K. Shin, J. S. Cho, S. K. Lee, M. H. Won, J. W. Kim, J. H. Park, *J. Med. Food* **2006**, *9*, 293. doi:10.1089/JMF.2006.9.293

[46] J. J. Ochoa, A. J. Farquharson, I. Grant, L. E. Moffat, S. D. Heys, K. W. Wahle, *Carcinogenesis* **2004**, *25*, 1185. doi:10.1093/CARCIN/BGH116
[47] A. Miller, C. Stanton, R. Devery, *Lipids* **2001**, *36*, 1161. doi:10.1007/S11745-001-0827-0
[48] S. C. Degner, M. Q. Kemp, G. T. Bowden, D. F. Romagnolo, *J. Nutr.* **2006**, *136*, 421.
[49] J. H. Kim, N. E. Hubbard, V. Ziboh, K. L. Erickson, *Biochim. et Biophys. Acta* **2005**, *1736*, 244. doi:10.1016/J.BBALIP.2005.08.015
[50] H. J. Song, A. A. Sneddon, S. D. Heys, K. W. Wahle, *Prostate* **2006**, *66*, 839. doi:10.1002/PROS.20351
[51] H. J. Cho, E. J. Kim, S. S. Lim, M. K. Kim, M. K. Sung, J. S. Kim, J. H. Park, *J. Nutr.* **2006**, *136*, 893.
[52] N. S. Kelley, N. E. Hubbard, K. L. Erickson, *J. Nutr.* **2007**, *137*, 2599.