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Chemo-Enzymatic Synthesis of Mixed Docosahexaenoic Acid Phosphatidylcholine Conjugates

Jordan Lehoux,^[a] Felix Grosjean,^[a] Alexandre Lobo,^[a] Michel Lagarde,^[b]
Nathalie Bernoud-Hubac,^[b] Thierry Durand,^[a] and Celine Crauste*^[a]

Docosahexaenoic acid (DHA, C22:6 n-3) is a critical omega-3 polyunsaturated fatty acid (PUFA) with essential roles in brain and retinal functions. Its selective uptake into these tissues is mediated by several pathways, including the transporter Mfsd2a, which preferentially utilizes lysophosphatidylcholine-DHA (LPC-DHA) as a substrate. Due to the low *de novo* synthesis of DHA, optimizing its dietary intake and exploring novel delivery strategies are important in pathologies that affect PUFA proportion. The present work focuses on developing an efficient chemo-enzymatic methodology to access structured mixed DHA phosphatidylcholines (mixed DHA-PCs), valuable biological tools for enhancing drug and/or DHA

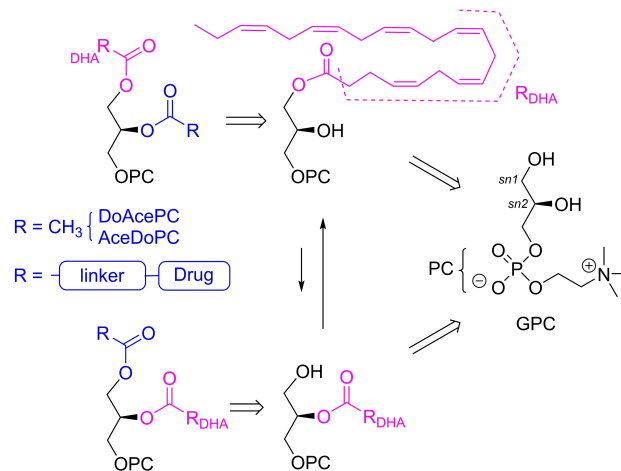
delivery to the brain and retina. Chemo-enzymatic methods were established to synthesize mixed *sn1*- or *sn2*-DHA-PC isomers, to study the importance of DHA position, from cost-effective glycerophosphorylcholine (GPC). The approach aligns with green chemistry principles by leveraging lipase-catalyzed regioselective acylation and deacylation reactions, thus reducing the number of chemical steps compared to traditional methods. The resulting mixed PCs AceDoPC®, DoAcePC (stabilized forms of LPC-DHA), and phenolic-DHA-PCs were produced at a scale suitable for future *in vivo* studies, enabling investigations into the positional effect of DHA on brain and retinal uptake.

Introduction

Docosahexaenoic acid (DHA, C22:6 n-3) is an omega 3 polyunsaturated fatty acid (PUFA), that is selectively accreted and efficiently retained in brain and retina tissues. It plays a crucial role in proper brain and retinal function due to its high proportion in their membranes (membrane structure, signaling molecule, resolution of inflammation...).^[1] Since *de novo* synthesis of DHA is low, nutrition has fundamental impact on appropriated DHA levels, in both healthy individuals and particularly in patients affected by neurodegenerative diseases. In the last decade lysophosphatidylcholine of DHA (LPC-DHA) has been identified as substrate of the transporter Mfsd2a,^[2] (member of the major facilitator superfamily), making LPC-DHA a preferential form of active DHA transport within retinal and brain tissues.^[3-6]

Thus, LPC-DHA is currently investigated as a tool for DHA supplementation, as it facilitates a longer-term accumulation of DHA and brain uptake compared to unesterified DHA.^[7] It could also be considered to inspire new drug delivery strategies, using LPC-DHA conjugates for the transport of small molecule drugs across Blood-Brain Barrier or Blood-Retina-Barrier.^[8]

Lysophosphatidylcholines are phospholipids produced by the hydrolysis of one fatty ester from phosphatidylcholine.^[2,9] Depending on the lipase involved, the *sn1*-lyso and *sn2*-lyso forms can physiologically exist. Both isomers were used to investigate their role as a source of DHA in the brain.^[2,9] However *sn1*-lyso-*sn2*-acyl-phosphatidylcholine (*sn1*-lyso-PC) is not stable in physiological plasma and undergoes isomerization to *sn1*-acyl-*sn2*-lyso-PC due to the nucleophilicity of the primary alcohol function, ultimately reaching an equilibrium with a 25/75 (*sn1*-lyso/*sn2*-lyso) ratio.^[10] In previous work, the team of Lagarde and co-workers developed a structured phospholipid called AceDoPC®,^[11] (Scheme 1) mimicking a stabilized form of *sn1*-lyso-*sn2*-DHA-PC, using an acetyl protecting group at the *sn1* position to prevent the isomerization process. AceDoPC® has been proved to efficiently and specifically deliver DHA to



Scheme 1. Chemo-enzymatic pathways of mixed DHA-PCs.

[a] J. Lehoux, F. Grosjean, A. Lobo, T. Durand, C. Crauste
IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France
E-mail: celine.crauste@umontpellier.fr

[b] M. Lagarde, N. Bernoud-Hubac
INSA Lyon, CNRS, LAMCOS, UMR5259, 69621 Villeurbanne, France

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the brain leading to DHA incorporation into specific brain regions that play key roles in memory, thoughts and cognitive functions.^[12] However, since DoAcePC (with DHA at the *sn1* position, Scheme 1) has never been synthesized, the importance of DHA at the *sn1* or *sn2* position in LPC transport has not been fully addressed. In this context, with an interest in both the delivery of DHA and active pharmaceutical drug to brain and retina tissues, we proposed to develop chemo-enzymatic synthesis of mixed structured phospholipids to achieve the following objectives: first, to achieve both AceDoPC® and DoAcePC isomers; second, to develop LPC-DHA drug conjugates (using a phenolic drug as an example) with DHA positioned at either the *sn1* or *sn2* position.

The chemical synthetic strategies to access structured phospholipid have been widely described over the past decade. Usual strategies starting from unphosphorylated enantiomerically pure glycerol derivatives are suboptimal for mixed PC synthesis intended for *in vivo* evaluation, due to the tedious protection–deprotection steps involved. In addition, most of them described the introduction of the fatty acid at the beginning of the strategy,^[13] which is not adapted to the weak stability of highly oxidizable PUFA such as DHA. Recent methodology developed by Papangelis *et al.*^[14] led to efficient synthesis of mixed PC with late fatty acid introduction, in 10 steps. However, the use of TFA treatment in this approach is not suitable due to the instability of DHA. The most widely reported and utilized stereoselective methodology for mixed PC to date was developed by Fasoli *et al.*,^[15] through a tin-mediated stereoselective *sn1* monoacylation of unexpensive starting L- α -glycerophosphorylcholine (GPC). However, traces of metal were reported in the final phospholipids, making them incompatible with *in vivo* assays.

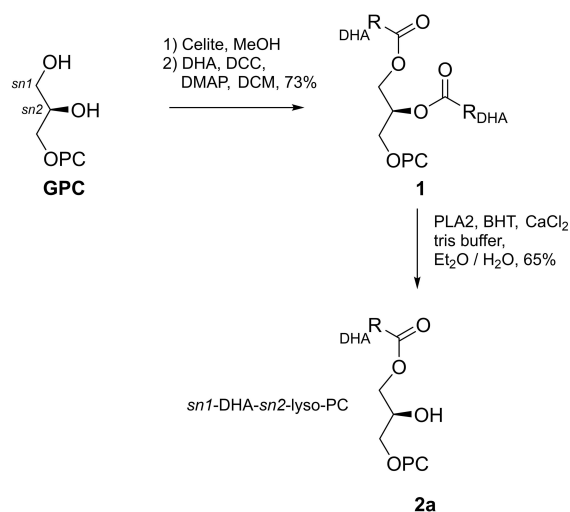
Starting from GPC or commercially available symmetrical PC, the enzymatic synthesis of lyso and mixed PC have also been reported. These enzymatic pathways have several advantages over chemical methods, the most important being the reduction in the number of steps required, aligning with the principles of green chemistry. To access *sn1*-lyso-PC, lipases *Rhizomucor miehei*, *Mucor javanicus*, *Rhizopus oryzae* or *Candida antartica*^[16–19] have been investigated for their ability to selectively deacylate saturated fatty acid at the *sn1* position of symmetrical PC. Regioselective acylation of GPC at *sn1* position was also reported using large excess of vinyl ester of saturated or mono unsaturated fatty acid.^[20,21] However, only a few studies described the use of enzymatic lipase to access more flexible and less stable DHA-phosphatidylcholine (DHA-PC). Polette *et al.*^[11] described the regioselective synthesis of *sn1*-lyso-*sn2*-DHA-PC starting from expensive *sn1*-stearoyl-*sn2*-DHA-PC using a lipase from *Rhizopus arrhizus*, while Zerouga *et al.*^[22] described the one of *sn1*-DHA-*sn2*-lyso-PC using honeybee venom Phospholipase A₂ (PLA₂, a selective *sn2* phospholipase), without information on final proportion of isomerization. Unfortunately, these enzymatic reactions are often described on a small analytical scale (less than 10 mg), with limited details on yield, analytical purity or isomerization. Developing an efficient synthetic methodology to access the mixed phosphatidylcholine containing DHA is therefore a scientific objective of

particular interest. In the present work, we propose developing a chemo-enzymatic synthesis of mixed *sn1*- or *sn2*-DHA-PC starting from inexpensive GPC. To conduct *in vivo* evaluations and confirm the impact of DHA positioning within these structured phospholipids, the synthesis scale was adjusted to produce quantities exceeding the typical analytical amounts observed in the enzymatic synthesis of such molecules. Since isomerizable LPC-DHA intermediates are involved in these strategies (Scheme 1), the analysis of regioisomer proportions was conducted using NMR or HPLC to report on acyl migration.

Results and Discussion

Mixed DHA-PC: DHA at the *sn1* Position

To access mixed PC having DHA at the *sn1* position with a limited number of steps, a chemo-enzymatic pathway was initially proposed, starting with the synthesis of *sn1*-DHA-*sn2*-lyso-PC **2a** from the commercially available GPC. Our first attempts to regioselectively acylate the *sn1* position of GPC with *Candida antartica* lipase B (CALB)^[21] failed using vinyl ester of PUFA. Synthesis of **2a** was thus realized in two steps as described in Scheme 2. GPC was first acylated with DHA in both *sn1* and *sn2* positions using DCC/DMAP coupling reagents to access di-DHA-PC **1**. To overcome insolubility of GPC in organic solvent, ensure good reproducibility and efficient yield of this reaction, the GPC was previously complexed on hyflo supercel Celite®.^[23] This methodology was preferred over cadmium salt^[24] to avoid metal traces in final products. Regioselective cleavage at the *sn2* position was then conducted using the *sn2* selective PLA₂ lipase. This reaction was performed using tris buffer and CaCl₂ necessary for enzyme activity.^[22] The reaction time had to be increased to 24 h to reach completion using DHA in comparison to less unsaturated linoleic acid (data not shown). To scale up this reaction from the analytical to larger scale (500 mg starting material), BHT was added to avoid DHA oxidation, and the work-up was optimized to allow the



Scheme 2. Chemo-enzymatic synthesis of *sn1*-DHA-*sn2*-lyso-PC **2a**.

challenging recovery of the zwitterionic *sn1*-DHA-*sn2*-lyso-PC **2a** (Table 1). Among the methodologies tested, the Bligh and Dyer process initially used for lipid extraction in biological media^[25] was the most efficient process for eliminating tris buffer and ensuring good recovery of **2a** in organic phase.

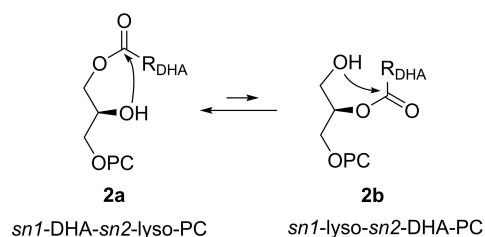
As previously mentioned, an isomerization can occur between the *sn1* and *sn2* LPC forms due to acyl group migration (Scheme 3). Although the equilibrium chemically favors the *sn2*-lyso-PC isomer, approximately 12% of the *sn1*-

lyso-PC isomer **2b** was observed, as shown by HPLC-UV and NMR analysis of the isolated **2a** (Figure 1, Table 2).

sn1-DHA-*sn2*-Ac-PC (DoAcePC, compound **3a**, Scheme 4) was synthesized by acetylation of **2a** following the conditions developed by Chupin *et al.*^[26] to provide the desired acetylated compound. In this reaction, DMAP was used as a catalyst to prevent the transfer of the phosphatidylcholine head group on the *sn2* position which could occur when stoichiometric amounts of base such as triethylamine are used. The proportion of DHA migration did not increase during the acetylation reaction (Figure S5–S7, Table 2). Several tens of milligrams of DoAcePC **3a** were obtained in only three steps.

To evaluate the potency of LPC-DHA as a chemical drug transporter for targeting brain or retinal tissue, the antioxidant and anticarbonyl stress^[27] agent isopropyl-phloroglucinol (*i*-Pr-Phloro) was selected and incorporated at the *sn2* position (Scheme 4). This isopropyl phloroglucinol derivative linked to DHA has previously shown *in vivo* protection of photoreceptor cells under light induced degeneration processes,^[28,29] and would be a perfect candidate to evaluate the potency of LPC-DHA as a retinal drug delivery system. Glutaryl isopropyl phloroglucinol **4** was synthesized from triisopropylsilyl (TIPS) *i*-Pr-phloroglucinol.^[30] A three-carbon chain length was selected because it prevents linker hydrolysis by intramolecular cyclization, as observed with a succinyl linker. Classical Steglich coupling^[31] allows incorporation of the phenolic scaffold at the *sn2* position of **2a**. Slow kinetic and moderate yield were observed, likely due to steric hindrance to access the *sn2* position. The TIPS protecting group of the resulting **5a** mixed PC was then removed using Et₃N-3HF reagent, which is compatible with the stability of both DHA and ester bond. The phenolic mixed DHA PC **6a** was obtained in 4 steps starting from GPC. Regarding the bulky mixed PCs **6**, in our hands, their separation by HPLC did not provide sufficient resolution to accurately quantify the proportion of isomerization. However, the **6a/6b** ratio could be estimated by comparing the integration of the CH₃ signals from the choline functional group (Figure 2, Table 2), the only ¹H NMR signal that differs between the two regioisomers.

Organic solvent used for liq/liq extraction during PLA2 hydrolysis work-up	Yield (2a)	Observations
AcOEt, pH 3, 0 °C	–	Degradation
AcOEt in continuous system 12 h, pH 3, 0 °C	–	Degradation
CH ₂ Cl ₂ /MeOH 1:1	> 100%	Presence of salts
1) CH ₂ Cl ₂ /MeOH (1/2), monophasic 2) CH ₂ Cl ₂ /H ₂ O (1/1) biphasic 3) CH ₂ Cl ₂ 4) CH ₂ Cl ₂ /MeOH/Brine 2/1/1	65%	Pure compound isolated



Scheme 3. LPC-DHA isomerization.

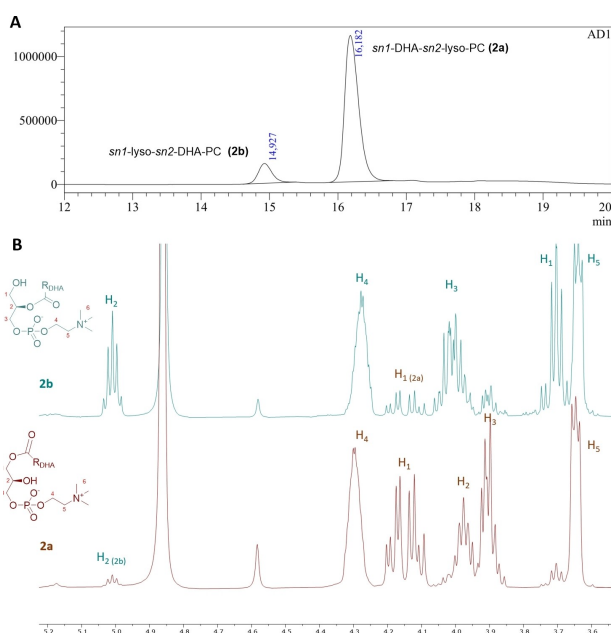
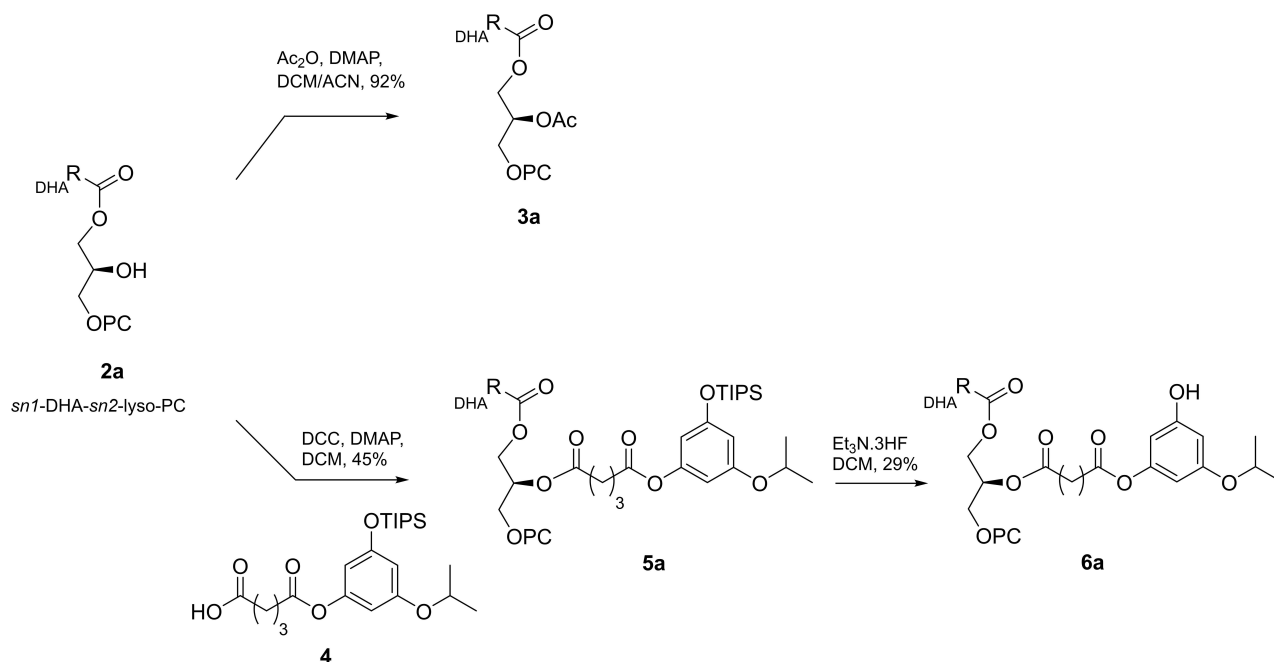


Figure 1. Isomerization of isolated **2a**: A) HPLC-UV analysis; B) Comparison of ¹H NMR signals of GPC part in **2a** and **2b** isomers.

Synthesis of	Isomer proportion (HPLC-UV)	Isomer proportion (¹ H NMR)	¹ H NMR signals compared ^[a]
2a	12%	13%	H ₂ (2a) vs H ₁ (2b)
2b	13%	18%	H ₁ (2b) vs H ₂ (2a)
3a	11%	11%	CH ₃ (Ac) (3a) vs (3b)
3b	14%	14%	CH ₃ (Ac) (3b) vs (3a)
6a	nd	17%	N(CH ₃) ₃ (6a) vs (6b)
6b	nd	5%	N(CH ₃) ₃ (6b) vs (6a)

[a] see Figure 1, S7 and 2.



Scheme 4. Chemical synthesis of Mixed DHA PC **3a** and **6a**.

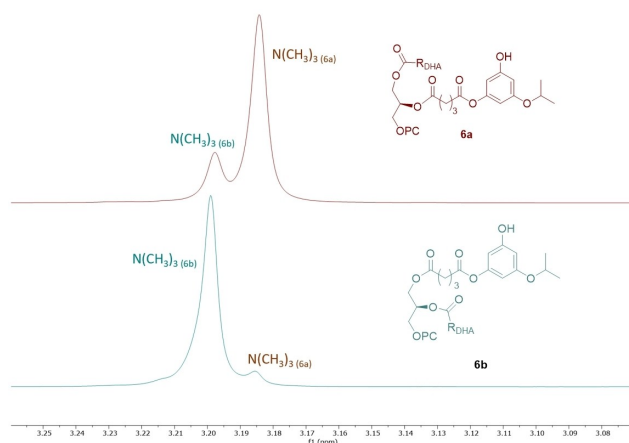


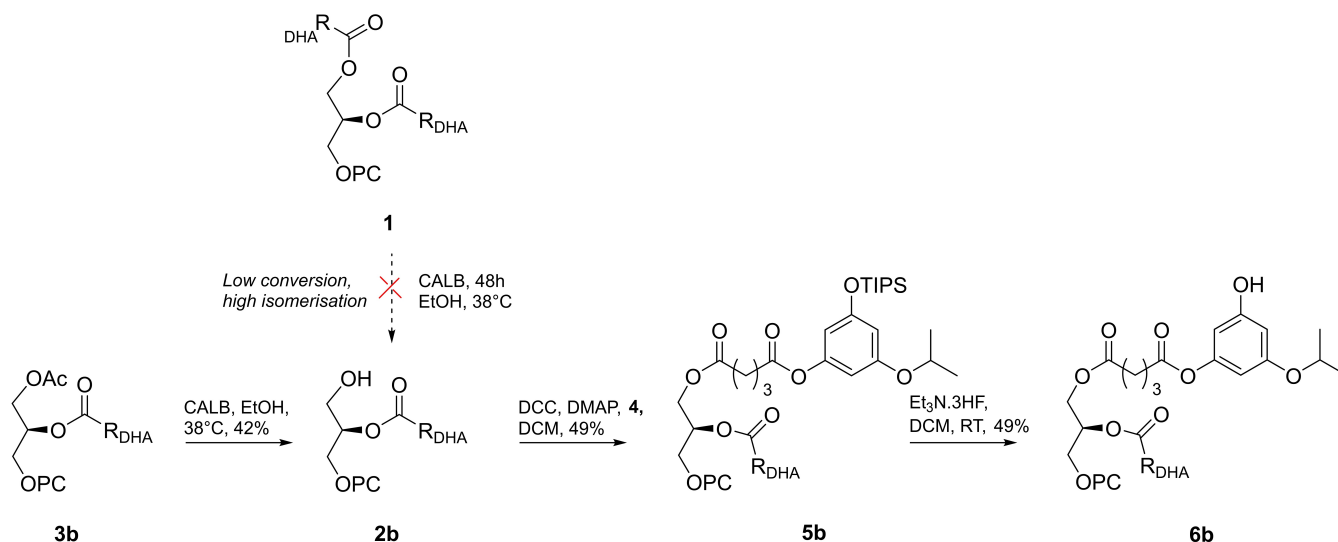
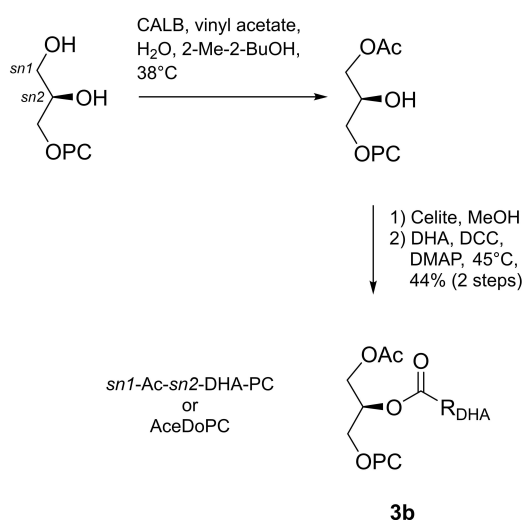
Figure 2. Comparison of ^1H NMR signals of $\text{N}(\text{CH}_3)_3$ in **6a** and **6b** isomers.

Mixed DHA-PC: DHA at the *sn2* Position

To access mixed-PC containing DHA at the *sn2* position the chemo-enzymatic pathway starting from GPC and utilizing LPC-DHA **2** as key intermediate was maintained. First attempts to synthesize *sn1*-lyso-*sn2*-DHA-PC **2b** was envisaged through regio-enzymatic deacylation at the *sn1* position of di-DHA-PC **1** (Scheme 5). The immobilized lipases CALB and Lipozyme® (*Mucor miehei*) were considered for selective deacylation, following the procedure developed by Yasuda *et al.*^[17] on soybean PC mixtures. However, enzymatic hydrolysis of DHA at the *sn1* position was significantly slower than for saturated^[30] or less unsaturated fatty acids, leaving over 50% of the starting material unreacted after 48 hours, along with notable isomerization. Acetyl group was thus used as a transient *sn1* protecting group during the synthesis of mixed PCs with DHA

at the *sn2* position, making AceDoPC® **3b** a key intermediate (Scheme 5). AceDoPC® **3b** was obtained starting from GPC in two steps (Scheme 6). To reduce DHA migration, acetyl was first introduced at the *sn1* position of GPC using supported CALB in 2-methyl-2-butanol. To set-up the enzymatic reaction at the gram scale compared to previous analytical scale procedure,^[12,32] the reaction conversion was followed by HPLC-MS analysis (no separation possible on TLC). Acetylation conversion was achieved after 18–24 h with no further progression over the following 3 days (around 70–80% estimated by both HPLC-MS and NMR performed on isolated product, see Figure S1 and S1). Due to weak affinity for organic solvent *sn1*-Ac-*sn2*-lyso-PC was then adsorbed on Celite®, as previously performed for GPC, to realize DHA coupling at the *sn2* position using DCC/DMAP reagents. Best coupling yield was obtained at a maximum temperature of 45 °C to avoid DHA degradation. Final purification yielded more than 500 mg of AceDoPC® **3b** in 44% overall yield, over two steps. Again, despite the unfavorable acyl migration from the *sn1* to the *sn2* position during both the acetylation and the DHA coupling, a final proportion of around 14% of the regioisomer DoAcePC **3a** was observed, as indicated by NMR and HPLC analysis (Figures S6–S7, Table 2).

AceDoPC® **3b** was then used as intermediate in mixed DHA-PC synthesis (Scheme 5). Acetyl cleavage was tested using regioselective lipases, Lipozyme® and CALB. The supported lipase from *Candida antartica* was more efficient leading to a complete conversion after 36 h of reaction. The *sn1*-lyso-*sn2*-DHA-PC **2b** was isolated in only 42% yield, likely due to acyl migration, which led, after lipase hydrolysis, to the starting GPC. A final isomerization proportion of approximately 13% was observed after purification, attributed to the higher reactivity of

Scheme 5. Chemical synthesis of mixed DHA-PC **6b**.Scheme 6. Synthesis of AceDoPC® **3b**.

the *sn1* hydroxyl group. *Sn1*-lyso-*sn2*-DHA-PC **2b** was then coupled to **4** and deprotected as previously performed, to afford the **6b** mixed *i*-Pr-phloroglucinol-DHA-PC. Surprisingly, probably due to the lower reactivity at the *sn2* compared to the *sn1* position during Steglich coupling, the final proportion of the regioisomer **6a**, coming from *sn2* acylation, reached only 5% (Figure 2, Table 2).

Conclusions

In conclusion, this study presents an original and flexible approach to the synthesis of mixed DHA-PCs, with highly oxidizable DHA positioned in either the *sn1* or the *sn2* position. Both bulky (phenol) and small (acetate) substituents were successfully incorporated as second acyl chains, broadening the structural diversity of mixed DHA-PCs with this methodology.

This work highlights the isomerization proportions, even when chromatographic separation was challenging, demonstrating the value of NMR as an alternative analytical tool. Importantly, such isomerization rates observed for the LPC derivatives align with those reported in comparable recent chemical strategies involving more stable fatty acids^[14] highlighting the value of such a chemo-enzymatic methodology. This approach is further reinforced by the reduced number of steps, as well as the use of less toxic reagents and the improved atom economy enabled by enzymatic pathways. New DHA-PC conjugates, such as phenolic DHA-PC and DoAcePC, have been synthesized for the first time. These final mixed DHA-PCs hold significant promise as biological tools for probing the roles of DHA and LPC-DHA in physiological processes, including their transport via the Mfsd2a transporter. The developed chemo-enzymatic pathways will contribute to the advancement of DHA-based therapeutics and brain/retina-targeted drug delivery systems by facilitating access to mixed DHA-PCs. Future work should focus on refining these methods to enhance scalability, while also exploring the role of DHA's positional specificity in phospholipids, and the potential of LPC-DHA as targeted vector for the brain or retina.

Experimental Section

General Experimental Information

All solvents were anhydrous reagents from commercial sources. *Candida Antarctica* Lipase B (CALB) was purchased from Sigma-Aldrich, GPC from Fluorochem (98%), Lipozyme® from Creative Enzymes, DHA from BLDpharm (95%). Unless otherwise noted, all chemicals and reagents were obtained commercially and used without purification. The reactions were monitored using TLC on plates that were pre-coated with silica gel 60 (Macherey-Nagel). The reaction components were visualized using a 254 nm UV lamp, stained with acidic p-anisaldehyde solution, KMNO₄ solution or phosphomolybdic acid solution. Purifications of the synthesized compounds were performed by column chromatography on silica

gel 40–63 μm . High resolution mass spectrometry (HRMS) was recorded using electrospray ionization (ESI) on Q-TOF mass spectrometer. NMR spectra were recorded at 400 or 500 MHz (^1H) and 101 or 126 MHz (^{13}C) using Bruker spectrometers. Chemical shifts are reported in parts per million (ppm, δ) relative to residual deuterated solvent peaks. The NMR spectra were assigned with the help of 2D NMR analyses (COSY, HSQC, and HMBC). The multiplicities reported are as follows: br=broad singlet, m=multiplet, s=singlet, d=doublet, t=triplet, q=quadruplet, quint=quintuplet, h=hexuplet, or combinations thereof. For the peak assignments, the following abbreviations were used: Ar=aromatic, CH=CH=aliphatic alkene, *i*-Pr=isopropyl, TIPS=triisopropylsilyl.

(R)-2,3-bis(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate (1). L- α -glycerophosphorylcholine GPC (400 mg, 1.56 mmol, 1 equiv.) was dissolved in 5 mL of MeOH and then added dropwise to Celite® hyflo supercel (1.2 g) in a teflon round bottom flask. The solvent was removed under reduced pressure and the GPC-Celite® complex was dried under vacuum at 80 °C in the presence of P_2O_5 for 12 hours. The complex was then suspended in 50 mL dry CH_2Cl_2 . DMAP (380 mg, 3.12 mmol, 2 equiv.), DCC (1.29 g, 6.24 mmol, 4 equiv.) and DHA (2.04 g, 6.24 mmol, 4 equiv.) were successively added and the mixture was stirred at 45 °C under nitrogen and in the dark for 72 hours. After cooling, the mixture was filtered over Celite®, then Dowex 50wx8 resin was added to the filtrate and stirred for 30 minutes. Filtration of the resin over Celite® and concentration of the filtrate under vacuum afforded the crude product as an orange oil. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/ H_2O 70/25/2 as eluent to yield the desired di-DHA-PC 1 (1 g, 1.14 mmol, 73%) as a greasy yellow solid. Rf = 0.5 (DCM/MeOH/ H_2O 70/25/2); ^1H NMR (400 MHz, CDCl_3): δ 5.42–5.27 (m, 24H, CH=CH_{DHA}), 5.24–5.18 (m, 1H, CH_{sn2}), 4.39 (dd, $^2J_{\text{H,H}}=12.0$ Hz, $^3J_{\text{H,H}}=3.1$ Hz, 1H, CH_{sn1a}), 4.33–4.26 (m, 2H, PO-CH₂-CH₂-N), 4.14 (dd, $^2J_{\text{H,H}}=12.1$ Hz, $^3J_{\text{H,H}}=7.1$ Hz, 1H, CH_{sn1b}), 4.03–3.88 (m, 2H, CH_{2sn3}), 3.82–3.70 (m, 2H, CH₂-N), 3.33 (s, 9H, N-(CH₃)₃), 2.90–2.78 (m, 20H, CH_{2DHA(bis-allylic)}), 2.39–2.34 (m, 8H, CH_{2DHA}), 2.10–2.03 (m, 4H, CH₂-CH_{3DHA}), 0.96 (t, $^3J_{\text{H,H}}=7.5$ Hz, 6H, CH_{3DHA}); ^{13}C NMR (101 MHz, CDCl_3): δ 173.0, 172.7, 132.2 (2 C), 129.5 (2 C), 128.7 (2 C), 128.5 (2 C), 128.5 (4 C), 128.2 (4 C), 128.2 (2 C), 128.0 (2 C), 127.9 (2 C), 127.2 (2 C), 70.8 (d, $^2J_{\text{C,P}}=7.8$ Hz), 66.7, 63.6 (d, $^2J_{\text{C,P}}=4.8$ Hz), 63.2, 59.4 (d, $^2J_{\text{C,P}}=4.6$ Hz), 54.7 (3 C), 34.3, 34.1, 25.8 (8 C), 25.7 (2 C), 22.7, 22.7, 20.7 (2 C), 14.4 (2 C); ^{31}P NMR (162 MHz, CDCl_3): δ -0.73; HRMS (TOF ESI+) m/z: calcd for $\text{C}_{52}\text{H}_{81}\text{NO}_8\text{P}$: 878.5694 [M+H]⁺, found: 878.5699 [M+H]⁺.

(R)-3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)-2-hydroxypropyl (2-(trimethylammonio)ethyl) phosphate (2a). Di-DHA-PC 1 (580 mg, 0.66 mmol, 1 equiv.) was dissolved in 40 mL Et₂O and 100 mL aqueous buffer (50 mM tris, 25 mM NaCl, 5 mM CaCl₂). 10 mL of a 0.5% BHT solution in MeOH were added, followed by bee venom PLA₂ (0.58 mg, 580 μL of a 1 mg/mL aqueous solution). The mixture was then stirred at 30 °C under nitrogen in the dark for 24 hours. The Et₂O was evaporated under vacuum and the extraction of *sn*1-DHA-lyso-PC was achieved based on the Bligh and Dyer method. First 100 mL DCM and 200 mL MeOH were added to the mixture and stirred for 15 minutes (monophasic). Then, 100 mL DCM and 100 mL H₂O were added (biphasic). The mixture was extracted with 6×100 mL DCM. Brine (50 mL) was then added to the aqueous phase and was extracted with 2×150 mL DCM/MeOH 2/1. The organic phases were gathered, dried over Na₂SO₄ and concentrated under vacuum. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/ H_2O 70/25/4 as eluent to yield the desired product 2a (243 mg, 0.43 mmol, 65%) as a greasy yellow solid. HPLC-UV analysis shows

around 12% of regioisomer 2b. Rf=0.1 (DCM/MeOH/ H_2O 70/25/2); ^1H NMR (400 MHz, CD_3OD): δ 5.42–5.27 (m, 12H, CH=CH_{DHA}), 4.33–4.26 (m, 2H, PO-CH₂-CH₂-N), 4.18 (dd, $^2J_{\text{H,H}}=11.4$ Hz, $^3J_{\text{H,H}}=4.5$ Hz, 1H, CH_{sn1a}), 4.11 (dd, $^2J_{\text{H,H}}=11.4$ Hz, $^3J_{\text{H,H}}=6.2$ Hz, 1H, CH_{sn1b}), 3.97 (p, $^3J_{\text{H,H}}=5.5$ Hz, 1H, CH_{sn2}), 3.92–3.87 (m, 2H, CH_{2sn3}), 3.66–3.63 (m, 2H, CH₂-N), 3.23 (s, 9H, N-(CH₃)₃), 2.88–2.81 (m, 10H, CH_{2DHA(bis-allylic)}), 2.41–2.36 (m, 4H, CH_{2DHA}), 2.09 (p, $^3J_{\text{H,H}}=7.4$ Hz, 2H, CH₂-CH_{3DHA}), 0.97 (t, $^3J_{\text{H,H}}=7.6$ Hz, 3H, CH_{3DHA}); ^{13}C NMR (101 MHz, CD_3OD): δ 174.6, 132.8, 130.3, 129.5, 129.2 (3 C), 129.1 (4 C), 128.9, 128.2, 69.8 (d, $^3J_{\text{C,P}}=7.3$ Hz), 67.8 (d, $^2J_{\text{C,P}}=5.5$ Hz), 67.5, 66.3, 60.4 (d, $^2J_{\text{C,P}}=4.4$ Hz), 54.7 (3 C), 34.9, 26.6 (3 C), 26.5, 26.4, 23.7, 21.5, 14.7; ^{31}P NMR (162 MHz, CD_3OD): δ -0.29; HRMS (TOF ESI+) m/z: calcd for $\text{C}_{30}\text{H}_{51}\text{NO}_7\text{P}$: 568.3398 [M+H]⁺, found: 568.3396 [M+H]⁺.

(R)-2-acetoxy-3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate (3a): *sn*1-DHA-*sn*2-lyso-PC 2a (30 mg, 0.05 mmol, 1 equiv.) was dissolved in MeCN (700 μL) and DCM (1 mL) under N₂. DMAP (3 mg, 0.02 mmol, 0.5 equiv.) and Ac₂O (59 μL , 0.62 mmol, 12 equiv.) were added to the solution and the mixture was stirred at room temperature for 1 hour. The mixture was then diluted with 20 mL DCM, Dowex 50wx8 resin was added to the solution and stirred for 30 minutes. Filtration of the resin over Celite® and concentration of the filtrate under vacuum afforded the crude product as an orange oil. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/ H_2O 70/25/4 as eluent to yield the desired DoAcePC 3a (30 mg, 0.05 mmol, 92%) as a greasy yellow solid. HPLC-UV analysis shows around 11% of regioisomer 3b. Rf=0.2 (DCM/MeOH/ H_2O 70/25/4); ^1H NMR (600 MHz, CD_3OD): δ 5.45–5.28 (m, 12H, CH=CH_{DHA}), 5.25–5.18 (m, 1H, CH_{2sn2}), 4.40 (dd, $^2J_{\text{H,H}}=12.0$ Hz, $^3J_{\text{H,H}}=3.6$ Hz, 1H, CH_{sn1a}), 4.31–4.25 (m, 2H, PO-CH₂-CH₂-N), 4.20 (dd, $^2J_{\text{H,H}}=12.0$ Hz, $^3J_{\text{H,H}}=6.6$ Hz, 1H, CH_{sn1b}), 4.03–4.00 (m, 2H, CH_{2sn3}), 3.66–3.64 (m, 2H, CH₂-N), 3.23 (s, 9H, N-(CH₃)₃), 2.88–2.81 (m, 10H, CH_{2DHA(bis-allylic)}), 2.41–2.37 (m, 4H, CH_{2DHA}), 2.04–2.13 (m, 5H, CH₂-CH_{3DHA}), 0.97 (t, $^3J_{\text{H,H}}=7.6$ Hz, 3H, CH_{3DHA}); ^{13}C NMR (151 MHz, CD_3OD): δ 174.3, 172, 132.8, 130.4, 129.5, 129.2, 129.2 (2 C), 129.1, 129.1 (2 C), 128.1, 128.9, 128.2, 72.0 (d, $^3J_{\text{C,P}}=8.8$ Hz), 67.4, 64.9 (d, $^2J_{\text{C,P}}=6.0$ Hz), 63.6, 60.5 (d, $^2J_{\text{C,P}}=5.8$ Hz), 54.7 (3 C), 34.8, 26.6 (2 C), 26.6, 26.5, 26.4, 23.7, 21.5, 21.0, 14.7; ^{31}P NMR (162 MHz, CD_3OD): δ -0.55; HRMS (TOF ESI+) m/z: calcd for $\text{C}_{32}\text{H}_{53}\text{NO}_8\text{P}$: 610.3509 [M+H]⁺, found: 610.3508 [M+H]⁺.

5-(3-isopropoxy-5-((triisopropylsilyl)oxy)phenoxy)-5-oxopentanoic acid (4): 3-isopropoxy-5-((triisopropylsilyl)oxy)phenol^[30] (1.32 g, 4.07 mmol, 1 equiv.) was dissolved in DCM (50 mL) under N₂, then pyridine (656 μL , 8.14 mmol, 2 equiv.) glutaric anhydride (927 mg, 8.14 mmol, 2 equiv.) and DMAP (99 mg, 0.81 mmol, 0.2 equiv.) were added to the solution. The mixture was stirred at 45 °C for 72 hours. The mixture was then quenched with 40 mL H₂O, extracted with 2×50 mL DCM, the organic phases were gathered, washed with brine, and dried over MgSO₄. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient pentane/EtOAc 90/10 to pentane/EtOAc 80/20 as eluent. Due to minor impurities the product was then recrystallized in pentane to yield compound 4 as a white solid (950 mg, 2.17 mmol, 53%). Rf=0.13 (cyclohexane/EtOAc 80/20); ^1H NMR (500 MHz, CDCl_3) δ 6.29 (t, $^4J_{\text{H,H}}=2.2$ Hz, 1H, H_{Ar}), 6.24 (t, $^4J_{\text{H,H}}=2.1$ Hz, 1H, H_{Ar}), 6.21 (t, $^4J_{\text{H,H}}=2.1$ Hz, 1H, H_{Ar}), 4.44 (hept, $^3J_{\text{H,H}}=6.1$ Hz, 1H, CH_{i-Pr}), 2.63 (t, $^3J_{\text{H,H}}=7.4$ Hz, 2H, CH₂-CO), 2.52 (t, $^3J_{\text{H,H}}=7.3$ Hz, 2H, CO-CH₂), 2.07 (quint, $^3J_{\text{H,H}}=7.3$ Hz, 2H, CO-CH₂-CH₂-CO), 1.31 (d, $^3J_{\text{H,H}}=6.1$ Hz, 6H, CH_{3-i-Pr}), 1.28–1.20 (m, 3H, CH_{TIPS}), 1.10 (s, 9H, CH_{3TIPS}), 1.09 (s, 9H, CH_{3TIPS}); ^{13}C NMR (126 MHz, CDCl_3) δ 178.2, 171.2, 159.3, 157.6, 152.0, 106.1, 105.6, 102.4, 70.4, 33.4, 32.8, 22.1 (2 C), 19.9, 18.0 (6 C), 12.7 (3 C); HRMS (TOF ESI-) m/z: calcd. for $\text{C}_{23}\text{H}_{37}\text{O}_6\text{Si}$ 437.2365 [M-H]⁻; found 437.2374 [M-H]⁻.

(R)-3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)-2-((5-(3-isopropoxy-5-((triisopropylsilyl)oxy)phenoxy)-5-oxopentanoyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate (5a): *sn*1-DHA-*sn*2-lyso-PC 2a (200 mg, 0.35 mmol, 1 equiv.) was dissolved in DCM (30 mL) under N₂. DCC (145 mg, 0.7 mmol, 2 equiv.), DMAP (42 mg, 0.35 mmol, 1 equiv.) and 4 (309 mg, 0.7 mmol, 2 equiv.) were then added. The mixture was stirred at 40 °C in the dark for 48 hours. After cooling, the mixture was filtered over Celite®, then Dowex 50wx8 resin was added to the filtrate and stirred for 30 minutes. Filtration of the resin over Celite® and concentration of the filtrate under vacuum afforded the crude product as an orange oil. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/H₂O 70/25/2 as eluent to yield the desired product 5a (154 mg, 0.16 mmol, 45%) as a greasy yellow solid. *R*_f = 0.24 (DCM/MeOH/H₂O 70/25/2); ¹H NMR (400 MHz, CD₃OD): δ 6.29 (d, ⁴J_{H,H} = 2.1 Hz, 2H, H_A), 6.22 (t, ⁴J_{H,H} = 2.0 Hz, 1H, H_A), 5.42–5.31 (m, 12H, CH = CH_{DHA}), 5.30–5.25 (m, 1H, CH_{sn2}), 4.50 (p, ³J_{H,H} = 6.0 Hz, 1H, CH_{i-Pr}), 4.44 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 3.3 Hz, 1H, CH_{sn1a}), 4.32–4.25 (m, 2H, PO-CH₂-CH₂-N), 4.21 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 6.9 Hz, 1H, CH_{sn1b}), 4.10–3.96 (m, 2H, CH_{2sn3}), 3.67–3.60 (m, 2H, CH₂-N), 3.21 (s, 9H, N-(CH₃)₃), 2.87–2.78 (m, 10H, CH_{2DHA(bis-allylic)}), 2.64 (t, ³J_{H,H} = 7.2 Hz, 2H, CH₂-CO), 2.50 (t, ³J_{H,H} = 7.3 Hz, 2H, CO-CH₂), 2.41–2.32 (m, 4H, CH_{2DHA}), 2.08 (p, ³J_{H,H} = 7.5 Hz, 1H, CH₂-CH_{3DHA}), 2.00 (quint, ³J_{H,H} = 7.1 Hz, 2H, CO-CH₂-CH₂-CO), 1.30 (d, ³J_{H,H} = 6.0 Hz, 6H, CH_{3i-Pr}), 1.29–1.20 (m, 3H, CH_{TPS}), 1.12 (d, ³J_{H,H} = 7.0 Hz, 18H, CH_{3TPS}), 0.97 (t, ³J_{H,H} = 7.6 Hz, 3H, CH_{3DHA}); ¹³C NMR (101 MHz, CD₃OD): δ 174.3, 173.9, 173.0, 160.6, 158.7, 153.6, 132.8, 130.4, 129.5, 129.2 (3 C), 129.1 (3 C), 129.0, 128.9, 128.2, 107.1, 106.2, 103.8, 72.1 (d, ³J_{C,P} = 8.3 Hz), 71.4, 67.5, 65.0 (d, ²J_{C,P} = 4.6 Hz), 63.7, 60.5 (d, ²J_{C,P} = 4.6 Hz), 54.7 (3 C), 34.9, 34.0, 34.0, 26.6 (4 C), 26.5, 23.7, 22.3 (2 C), 21.5, 21.1, 18.4 (6 C), 14.7, 13.9 (3 C); ³¹P NMR (162 MHz, CD₃OD): δ -0.64; HRMS (TOF ESI+) *m/z*: calcd for C₅₃H₈₇NO₁₂PSi: 988.5730 [M + H]⁺, found: 988.5740 [M + H]⁺.

(R)-3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)-2-((5-(3-hydroxy-5-isopropoxyphenoxy)-5-oxopentanoyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate (6a): 5a (145 mg, 0.15 mmol, 1 equiv.) was dissolved in DCM (6 mL) under N₂, and Et₃N·3HF (72 μL, 0.45 mmol, 3 equiv.) was added. The mixture was stirred at room temperature in the dark for 48 hours. The mixture was then concentrated under vacuum to afford the crude product as an orange oil. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/H₂O 70/25/2 as eluent to yield the desired mixed DHA-PC 6a (35 mg, 0.04 mmol, 29%) as a pale-yellow oil. *R*_f = 0.2 (DCM/MeOH/H₂O 70/25/2); ¹H NMR (400 MHz, CD₃OD): δ 6.23 (t, ³J_{H,H} = 2.2 Hz, 1H, H_A), 6.17–6.11 (m, 2H, H_A), 5.45–5.29 (m, 12H, CH = CH_{DHA}), 5.33–5.23 (m, 1H, CH_{sn2}), 4.50 (p, ³J_{H,H} = 6.0 Hz, 1H, CH_{i-Pr}), 4.43 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 3.5 Hz, 1H, CH_{sn1a}), 4.31–4.24 (m, 2H, PO-CH₂-CH₂-N), 4.22 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 6.8 Hz, 1H, CH_{sn1b}), 4.11–3.97 (m, 2H, CH_{2sn3}), 3.65–3.57 (m, 2H, CH₂-N), 3.18 (s, 9H, N-(CH₃)₃), 2.92–2.78 (m, 10H, CH_{2DHA(bis-allylic)}), 2.68–2.57 (m, 2H, CH₂-CO), 2.54–2.43 (m, 2H, CO-CH₂), 2.45–2.28 (m, 4H, CH_{2DHA}), 2.08 (p, ³J_{H,H} = 7.4 Hz, 2H, CH₂-CH_{3DHA}), 2.00 (p, ³J_{H,H} = 7.2 Hz, 2H, CO-CH₂-CH₂-CO), 1.29 (d, ³J_{H,H} = 6.0 Hz, 6H, CH_{3i-Pr}), 0.97 (t, ³J_{H,H} = 7.5 Hz, 3H, CH_{3DHA}); ¹³C NMR (101 MHz, CD₃OD): δ 174.3, 173.9, 173.2, 160.9, 160.2, 153.7, 132.8, 130.4, 129.5, 129.2 (3 C), 129.1 (3 C), 129.0, 128.9, 128.2, 102.6, 101.9, 101.7, 72.1 (d, ³J_{C,P} = 8.4 Hz), 71.2, 67.4, 65.1 (d, ²J_{C,P} = 4.8 Hz), 63.7, 60.5 (d, ²J_{C,P} = 4.6 Hz), 54.7, 54.7, 54.6, 34.8, 34.0, 33.9, 26.6 (4 C), 26.4, 23.7, 22.3 (2 C), 21.5, 21.2, 14.7; ³¹P NMR (162 MHz, CD₃OD): δ -0.29; HRMS (TOF ESI+) *m/z*: calcd for C₄₄H₆₇NO₁₂P: 832.4395 [M + H]⁺, found: 832.4399 [M + H]⁺.

(R)-3-acetoxy-2-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propyl (2-(trimethylammonio)ethyl) phosphate (3b): L-α-glycerophosphorylcholine GPC (1 g, 3.89 mmol, 1 equiv.) was dissolved in distilled H₂O (1.4 mL), 2-methyl-2-butanol (16 mL), vinyl acetate (14.3 mL, 155.5 mmol, 40 equiv.) and the supported CALB lipase (780 mg) was added. The reaction was followed by HPLC-MS analysis. The mixture was stirred at 38 °C at 150 rpm (rotavapor) for 18 hours. The mixture was then filtered on Celite®. The resin was washed three times with MeOH and the filtrate was concentrated under vacuum. Trituration in Acetone was realized and afforded the crude *sn*1-Ac-*sn*2-lyso-PC as an orange oil. NMR shows a mixture of the desired product and the starting GPC (approx. 73/27 ratio) and traces of acetic acid. ¹H NMR (400 MHz, CD₃OD): δ 4.34–4.24 (m, 2H, PO-CH₂-CH₂-N), 4.17 (dd, ²J_{H,H} = 11.3, ³J_{H,H} = 4.5 Hz, 1H, CH_{sn1a}), 4.10 (dd, ²J_{H,H} = 11.4, ³J_{H,H} = 6.1 Hz, 1H, CH_{sn1b}), 4.00–3.95 (m, 1H, CH_{sn2}), 3.94–3.85 (m, 2H, CH_{2sn3}), 3.67–3.62 (m, 2H, CH₂-N), 3.22 (s, 9H, N-(CH₃)₃), 2.06 (s, 3H, CH_{3Ac}); ³¹P NMR (162 MHz, CD₃OD): δ 0.02. Half of the crude is then engaged in the coupling step without further purification. The residue was dissolved in 5 mL of MeOH and then added dropwise to Celite® hyflo supercel (2 g) in a teflon flask. The solvent was removed under reduced pressure and the Celite® complex was dried under vacuum at 80 °C in the presence of P₂O₅ for 12 hours (acetic acid traces from the crude were removed at this step). The complex was then suspended in 40 mL dry CH₂Cl₂. DMAP (355 mg, 2.92 mmol, 1.5 equiv.), DCC (803 mg, 3.89 mmol, 2 equiv.) and DHA (1.28 g, 3.89 mmol, 2 equiv.) were successively added and the mixture was stirred at 45 °C under nitrogen and in the dark for 72 hours. After cooling, the mixture was filtered over Celite®, then Dowex 50wx8 resin was added to the filtrate and stirred for 30 minutes. Filtration of the resin over Celite® and concentration of the filtrate under vacuum afforded the crude product as an orange oil. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/H₂O 70/25/3 as eluent to yield the desired AceDoPC® 3b (520 mg, 0.85 mmol, 44% on two steps) as a greasy yellow solid. HPLC-UV analysis shows around 14% of regioisomer 3a. *R*_f = 0.2 (DCM/MeOH/H₂O 70/25/3); ¹H NMR (400 MHz, CD₃OD): δ 5.45–5.27 (m, 12H, CH = CH_{DHA}), 5.26–5.19 (m, 1H, CH_{sn2}), 4.39 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 3.6 Hz, 1H, CH_{sn1a}), 4.33–4.23 (m, 2H, PO-CH₂-CH₂-N), 4.18 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 6.5 Hz, 1H, CH_{sn1b}), 4.02–3.98 (m, 2H, CH_{2sn3}), 3.65–3.62 (m, 2H, CH₂-N), 3.22 (s, 9H, N-(CH₃)₃), 2.89–2.80 (m, 10H, CH_{2DHA(bis-allylic)}), 2.41–2.38 (m, 4H, CH_{2DHA}), 2.09 (p, ³J_{H,H} = 7.3 Hz, 2H, CH₂-CH_{3DHA}), 2.04 (s, 3H, CH_{3Ac}), 0.97 (t, ³J_{H,H} = 7.5 Hz, 3H, CH_{3DHA}); ¹³C NMR (75 MHz, MeOD): δ 174.0, 172.3, 132.8, 130.3, 129.4, 129.2 (2 C), 129.2 (2 C), 129.1 (2 C), 129.1, 128.9, 128.2, 121.8 (d, ³J_{C,P} = 8.3 Hz), 67.4, 64.8 (d, ²J_{C,P} = 5.5 Hz), 63.7, 60.5 (d, ²J_{C,P} = 5.0 Hz), 54.7, 54.7, 54.6, 35.1, 26.6 (2 C), 26.5 (2 C), 26.4, 23.7, 21.5, 20.7, 14.7; ³¹P NMR (162 MHz, CD₃OD): δ -0.57; HRMS (TOF ESI+) *m/z*: calcd for C₃₂H₅₃NO₈P: 610.3503 [M + H]⁺, found: 610.3508 [M + H]⁺.

(R)-2-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)-3-hydroxypropyl (2-(trimethylammonio)ethyl) phosphate (2b): AceDoPC® 3b (500 mg, 0.82 mmol, 1 equiv.) was dissolved in 10 mL EtOH 96%, CALB (200 mg, acrylic resin supported) was added and the mixture was stirred at 38 °C for 36 hours. The mixture was then filtered over Celite® and concentration of the filtrate under vacuum afforded the crude product as a yellow oil. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/H₂O 70/25/4 as eluent to yield the desired product 2b (195 mg, 0.34 mmol, 42%) as a pale-yellow oil. HPLC-UV analysis shows around 13% of regioisomer 2a. *R*_f = 0.18 (DCM/MeOH/H₂O 70/25/4); ¹H NMR (400 MHz, CD₃OD): δ 5.47–5.25 (m, 12H, CH = CH_{DHA}), 5.01 (p, ³J_{H,H} = 5.2 Hz, 1H, CH_{sn2}), 4.34–4.23 (m, 2H, PO-CH₂-CH₂-N), 4.07–3.94 (m, 2H, CH_{2sn3}), 3.73 (dd, ²J_{H,H} = 12.0, ³J_{H,H} = 5.0 Hz, 1H, CH_{sn1a}), 3.68 (dd, ²J_{H,H} = 12.1, ³J_{H,H} = 5.7 Hz, 1H, CH_{sn1b}),

3.67–3.61 (m, 2H, CH₂-N), 3.22 (s, 9H, N-(CH₃)₃), 2.93–2.79 (m, 10H, CH₂DHA(bis-allylic)), 2.48–2.33 (m, 4H, CH₂ DHA), 2.09 (p, ³J_{H,H} = 7.4 Hz, 2H, CH₂-CH₃ DHA), 0.97 (t, ³J_{H,H} = 7.5 Hz, 3H, CH₃DHA); ¹³C NMR (101 MHz, CD₃OD): δ 174.26, 132.81, 130.25, 129.47, 129.21 (3 C), 129.13 (4 C), 128.93, 128.19, 74.83 (d, ²J_{C,P} = 8.1 Hz), 67.48, 64.73 (d, ²J_{C,P} = 4.8 Hz), 61.22, 60.43 (d, ²J_{C,P} = 4.6 Hz), 54.69 (3 C), 35.04, 26.57 (4 C), 26.44, 23.70, 21.51, 14.67; ³¹P NMR (162 MHz, CD₃OD): δ -0.01; HRMS (TOF ESI+) m/z: calcd for C₃₀H₅₁NO₇P: 568.3403 [M + H]⁺, found: 568.3414 [M + H]⁺.

(R)-2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyloxy)-3-((5-(3-isopropoxy-5-

(triisopropylsilyloxy)phenoxy)-5-oxopentanoylethyl)propyl (2-(trimethylammonio)ethyl) phosphate (5b): *sn1-lyso-sn2-DHA-PC* 2b (180 mg, 0.32 mmol, 1 equiv.) was dissolved in DCM (30 mL) under N₂. DCC (131 mg, 0.63 mmol, 2 equiv.), DMAP (38 mg, 0.32 mmol, 1 equiv.) and 4 (278 mg, 0.63 mmol, 2 equiv.) were added. The mixture was stirred at 40 °C in the dark for 24 hours. After cooling, the mixture was filtered over Celite®, then Dowex 50wx8 resin was added to the filtrate and stirred for 30 minutes. Filtration of the resin over Celite® and concentration of the filtrate under vacuum afforded the crude product as an orange oil. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/H₂O 70/25/2 as eluent to yield the desired product 5b (153 mg, 0.15 mmol, 49%) as a greasy yellow solid. R_f = 0.24 (DCM/MeOH/H₂O 70/25/2); ¹H NMR (400 MHz, CD₃OD): δ 6.28 (d, ⁴J_{H,H} = 2.0 Hz, 2H, H_A), 6.21 (t, ⁴J_{H,H} = 2.0 Hz, 1H, H_A), 5.46–5.29 (m, 12H, CH = CH_{DHA}), 5.29–5.22 (m, 1H, CH_{sn2}), 4.50 (p, ³J_{H,H} = 6.1 Hz, 1H, CH_{i-Pr}), 4.46 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 3.4 Hz, 1H, CH_{sn1a}), 4.32–4.25 (m, 2H, PO-CH₂-CH₂-N), 4.21 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 6.7 Hz, 1H, CH_{sn1b}), 4.02 (t, ³J_{H,H} = 6.0 Hz, 2H, CH_{2sn3}), 3.67–3.60 (m, 2H, CH₂-N), 3.22 (s, 9H, N-(CH₃)₃), 2.92–2.78 (m, 10H, CH₂DHA(bis-allylic)), 2.62 (t, ³J_{H,H} = 7.3 Hz, 2H, CH₂-CO), 2.47 (t, ³J_{H,H} = 7.3 Hz, 2H, CO-CH₂), 2.45–2.31 (m, 4H, CH₂DHA), 2.08 (p, ³J_{H,H} = 7.3 Hz, 1H, CH₂-CH₃ DHA), 1.99 (p, ³J_{H,H} = 7.2 Hz, 2H, CO-CH₂-CH₂-CH₂-CO), 1.30 (d, ³J_{H,H} = 6.0 Hz, 6H, CH₃i-Pr), 1.29–1.20 (m, 3H, CH_{TIPS}), 1.12 (d, ³J_{H,H} = 6.9 Hz, 18H, CH₃TIPS), 0.97 (t, ³J_{H,H} = 7.5 Hz, 3H, CH₃DHA); ¹³C NMR (101 MHz, CD₃OD): δ 174.2, 174.0, 172.9, 160.6, 158.7, 153.6, 132.8, 130.4, 129.5, 129.2 (3 C), 129.1 (3 C), 129.0, 128.9, 128.2, 107.1, 106.2, 103.8, 72.9 (d, ²J_{C,P} = 8.3 Hz), 71.4, 67.5, 64.9 (d, ³J_{C,P} = 4.9 Hz), 63.8, 60.5 (d, ³J_{C,P} = 4.6 Hz), 54.7 (3 C), 35.0, 34.0, 34.0, 26.6 (4 C), 26.5, 23.7, 22.3 (2 C), 21.5, 21.1, 18.4 (6 C), 14.7, 13.9 (3 C). ³¹P NMR (162 MHz, CD₃OD): δ -0.63; HRMS (TOF ESI+) m/z: calcd for C₅₃H₈₇NO₁₂PSi: 988.5730 [M + H]⁺, found: 988.5728 [M + H]⁺.

(R)-2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyloxy)-3-((5-(3-hydroxy-5-isopropoxyphenoxy)-5-oxopentanoylethyl)propyl (2-(trimethylammonio)ethyl) phosphate (6b): 5b (145 mg, 0.15 mmol, 1 equiv.) was dissolved in DCM (6 mL) under N₂, and Et₃N·3HF (72 μL, 0.45 mmol, 3 equiv.) was added. The mixture was stirred at room temperature in the dark for 18 hours. The mixture was then concentrated under vacuum to afford the crude product as an orange oil. The crude was then purified through silica gel column chromatography (liquid deposit) with a gradient DCM/MeOH 80/20 to DCM/MeOH/H₂O 70/25/2 as eluent to yield the desired mixed DHA-PC 6b (60 mg, 0.07 mmol, 49%) as a pale-yellow oil. ¹H NMR analysis shows around 5% of regioisomer 6a. R_f = 0.2 (DCM/MeOH/H₂O 70/25/2); ¹H NMR (400 MHz, CD₃OD): δ 6.23 (t, ³J_{H,H} = 2.2 Hz, 1H, H_A), 6.18–6.10 (m, 2H, H_A), 5.67–5.29 (m, 12H, CH = CH_{DHA}), 5.28–5.22 (m, 1H, CH_{sn2}), 4.51 (p, ³J_{H,H} = 6.1 Hz, 1H, CH_{i-Pr}), 4.46 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 3.6 Hz, 1H, CH_{sn1a}), 4.32–4.24 (m, 2H, PO-CH₂-CH₂-N), 4.21 (dd, ²J_{H,H} = 12.0 Hz, ³J_{H,H} = 6.3 Hz, 1H, CH_{sn1b}), 4.03 (t, ³J_{H,H} = 6.0 Hz, 2H, CH_{2sn3}), 3.66–3.58 (m, 2H, CH₂-N), 3.20 (s, 9H, N-(CH₃)₃), 3.02–2.77 (m, 10H, CH₂DHA(bis-allylic)), 2.62 (t, ³J_{H,H} = 7.3 Hz, 2H, CH₂-CO), 2.48 (t, ³J_{H,H} = 7.2 Hz, 2H, CO-CH₂), 2.43–2.33 (m, 4H, CH₂DHA), 2.08 (p, ³J_{H,H} = 7.3 Hz, 2H, CH₂-CH₃ DHA), 1.99 (p,

³J_{H,H} = 7.2 Hz, 2H, CO-CH₂-CH₂-CH₂-CO), 1.29 (d, ³J_{H,H} = 6.0 Hz, 6H, CH₃i-Pr), 0.97 (t, ³J_{H,H} = 7.5 Hz, 3H, CH₃DHA); ¹³C NMR (101 MHz, CD₃OD): δ 174.2, 174.0, 173.1, 160.9, 160.2, 153.7, 132.8, 130.4, 129.5, 129.2 (3 C), 129.1 (3 C), 129.0, 128.9, 128.2, 102.6, 101.9, 101.7, 71.9 (d, ²J_{C,P} = 8.4 Hz), 71.2, 67.5, 64.8 (d, ³J_{C,P} = 4.8 Hz), 63.7, 60.5 (d, ³J_{C,P} = 4.6 Hz), 54.7 (3 C), 35.0, 34.0, 33.8, 26.6 (4 C), 26.4, 23.7, 22.3 (2 C), 21.5, 21.2, 14.7; ³¹P NMR (162 MHz, CD₃OD): δ -0.75; HRMS (TOF ESI+) m/z: calcd for C₄₄H₆₇NO₁₂P: 832.4401 [M + H]⁺, found: 832.4373 [M + H]⁺.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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- [1] L. A. Horrocks, Y. K. Yeo, *Pharmacol. Res.* **1999**, *40*, 211–225.
- [2] L. N. Nguyen, D. Ma, G. Shui, P. Wong, A. Cazenave-Gassiot, X. Zhang, M. R. Wenk, E. L. Goh, D. L. Silver, *Nature* **2014**, *509*, 503–6.
- [3] R. P. Bazinet, N. Bernoud-Hubac, M. Lagarde, *Prostaglandins Leukot. Essent. Fatty Acids* **2019**, *142*, 1–3.
- [4] B. H. Wong, J. P. Chan, A. Cazenave-Gassiot, R. W. Poh, J. C. Foo, D. L. Galam, S. Ghosh, L. N. Nguyen, V. A. Barathi, S. W. Yeo, C. D. Luu, M. R. Wenk, D. L. Silver, *J. Biol. Chem.* **2016**, *291*, 10501–14.
- [5] B. H. Wong, D. L. Silver, *Adv. Exp. Med. Biol.* **2020**, *1276*, 223–234.
- [6] N. Bernoud, L. Fenart, P. Molière, M.-P. Dehouck, M. Lagarde, R. Cecchelli, J. Lecerf, *J. Neurochem.* **1999**, *72*, 338–345.
- [7] D. Sugasini, R. Thomas, P. C. R. Yalagala, L. M. Tai, P. V. Subbaiah, *Sci. Rep.* **2017**, *7*, 11263.
- [8] J.-Z. Wang, N. Xiao, Y.-Z. Zhang, C.-X. Zhao, X.-H. Guo, L.-M. Lu, *Pharmacol. Res.* **2016**, *104*, 124–131.
- [9] F. Thies, C. Pillon, P. Molière, M. Lagarde, J. Lecerf, *Am. J. Physiol.* **1994**, *267*, R1273–9.
- [10] M. Croset, N. Brossard, A. Polette, M. Lagarde, *Biochem. J.* **2000**, *345 Pt 1*, 61–67.
- [11] A. Polette, C. Deshayes, B. Chantegrel, M. Croset, J. M. Armstrong, M. Lagarde, *Lipids* **1999**, *34*, 1333–1337.
- [12] M. Hachem, A. Gélouën, A. L. Van, B. Foumaux, L. Fenart, F. Gosselet, P. Da Silva, G. Breton, M. Lagarde, M. Picq, N. Bernoud-Hubac, *Mol. Neurobiol.* **2016**, *53*, 3205–3215.
- [13] R. Rosseto, N. Bibak, J. Hajdu, *Tetrahedron Lett.* **2004**, *45*, 7371–7373.
- [14] A. Papangelis, T. Ulven, *J. Org. Chem.* **2022**, *87*, 8194–8197.
- [15] E. Fasoli, A. Arnone, A. Caligiuri, P. D'Arrigo, L. de Ferra, S. Servi, *Org. Biomol. Chem.* **2006**, *4*, 2974–2978.
- [16] M. Balakrishna, S. S. Kaki, M. S. L. Karuna, S. Sarada, C. G. Kumar, R. B. N. Prasad, *Food Chem.* **2017**, *221*, 664–672.
- [17] S. Yasuda, Y. Yamamoto, *Biotechnol. Rep.* **2020**, *27*, e00505.
- [18] D. Adlercreutz, H. Budde, E. Wehtje, *Biotechnol. Bioeng.* **2002**, *78*, 403–411.

- [19] T. Morimoto, N. Mukarami, A. Nagatsu, J. Sakakibara, *Tetrahedron Lett.* **1993**, *34*, 2487–2490.
- [20] C. Virto, P. Adlercreutz, *Enzyme Microb. Technol.* **2000**, *26*, 630–635.
- [21] E. Garcia-Quinto, P. Garcia-Garcia, J. M. Guisan, G. Fernandez-Lorente, *Food Chem.* **2023**, *401*, 134109.
- [22] M. Zerouga, W. Stillwell, L. J. Janski, *Anti-Cancer Drugs* **2002**, *13*, 301.
- [23] K. Ichihara, H. Iwasaki, K. Ueda, R. Takizawa, H. Naito, M. Tomosugi, *Chem. Phys. Lipids* **2005**, *137*, 94–99.
- [24] J. S. Chadha, *Chem. Phys. Lipids* **1970**, *4*, 104–108.
- [25] E. G. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.
- [26] V. V. Chupin, O. V. Ostapenko, V. N. Klykov, M. V. Anikin, G. A. Serebrennikova, *Chem. Phys. Lipids* **1996**, *81*, 35–43.
- [27] H. M. Semchyshyn, *Sci. World J.* **2014**, *2014*, 417842.
- [28] N. Taveau, A. Cubizolle, L. Guillou, N. Pinquier, E. Moine, D. Cia, V. Kalatzis, J. Vercauteren, T. Durand, C. Crauste, P. Brabet, *Exp. Mol. Med.* **2020**, *52*, 1090–1101.
- [29] M. Vincent, L. Simon, P. Brabet, P. Legrand, C. Dorandeu, J. L. K. Him, T. Durand, C. Crauste, S. Begu, *Pharmaceutics* **2022**, *14*, 1029.
- [30] C. Crauste, C. Vigor, P. Brabet, M. Picq, M. Lagarde, C. Hamel, T. Durand, J. Vercauteren, *Eur. J. Org. Chem.* **2014**, *21*, 4548–4561.
- [31] B. Neises, W. Steglich, *Angew. Chem. Int. Ed* **1978**, *17*, 522–524.
- [32] A. Lo Van, B. Fourmaux, M. Picq, M. Guichardant, M. Lagarde, N. Bernoud-Hubac, *Lipids* **2017**, *52*, 751–761.

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