ORGANIC PROCESS RESEARCH & DEVELOPMENT
OPR&D

Full Paper

Scalable Synthesis and Purification of Acetylated Phosphatidyl Choline Headgroup

Rajesh Subramaniam, Ramesh Jagadeesan, Iswarya Mathew, Yana Cen, and Stefan Balaz

Org. Process Res. Dev., **Just Accepted Manuscript** • DOI: 10.1021/acs.oprd.6b00261 • Publication Date (Web): 24 Jan 2017

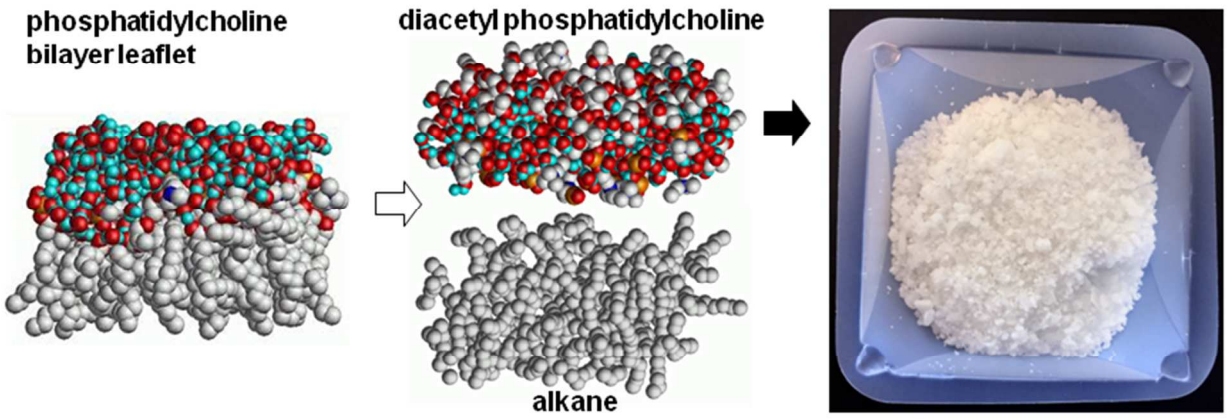
Scalable Synthesis and Purification of Acetylated Phosphatidyl Choline Headgroup

Rajesh Subramaniam,[†] *Ramesh Jagadeesan,*[‡] *Iswarya Mathew,*[†] *Yana Cen,*[†] *and Stefan Balaz*[*,†]

[†]Department of Pharmaceutical Sciences, Vermont Campus, Albany College of Pharmacy and Health Sciences, 261 Mountain View Drive, Colchester, Vermont 05446, Unites States

[‡]Pharmaceutical Development Unit, Kemwell Biopharma Pvt Ltd, 34 Tumkur Road, T-Begur, Nelamangala, Bangalore Rural- 562123, India

Table of Contents Graphic



ABSTRACT: The acetylated headgroup of the most abundant mammalian phospholipid, 1,2-diacetyl-3-*sn*-phosphatidyl choline (DAcPC), has several important applications in research. For instance, it can be dissolved in the same amount of water as in the fluid PC bilayer, to create a surrogate of a PC headgroup stratum, for studying solvation of small molecules and the influence of their structure on the process. In contrast to PC derivatives with longer acyl chains, DAcPC does not self-aggregate, rendering the aqueous solution homogeneous and suitable for simplified analyses of interactions of molecules with the headgroups. Several studies have been published where DAcPC was used in a crudely purified form. Here we describe a one-step preparation of DAcPC from commercially available bulk chemicals and purification of the product by crystallization and washing. The process gives a good yield and is easily scalable. The availability of enantiopure, crystalline DAcPC could open the door to more extensive biochemical, pharmacological, and nutritional studies of this interesting chemical.

KEYWORDS: phosphatidyl choline (PC) headgroup, PC headgroup stratum surrogate, drug-headgroup interactions, drug solvation in headgroup stratum, PC bilayer, bilayer accumulation, bilayer transport

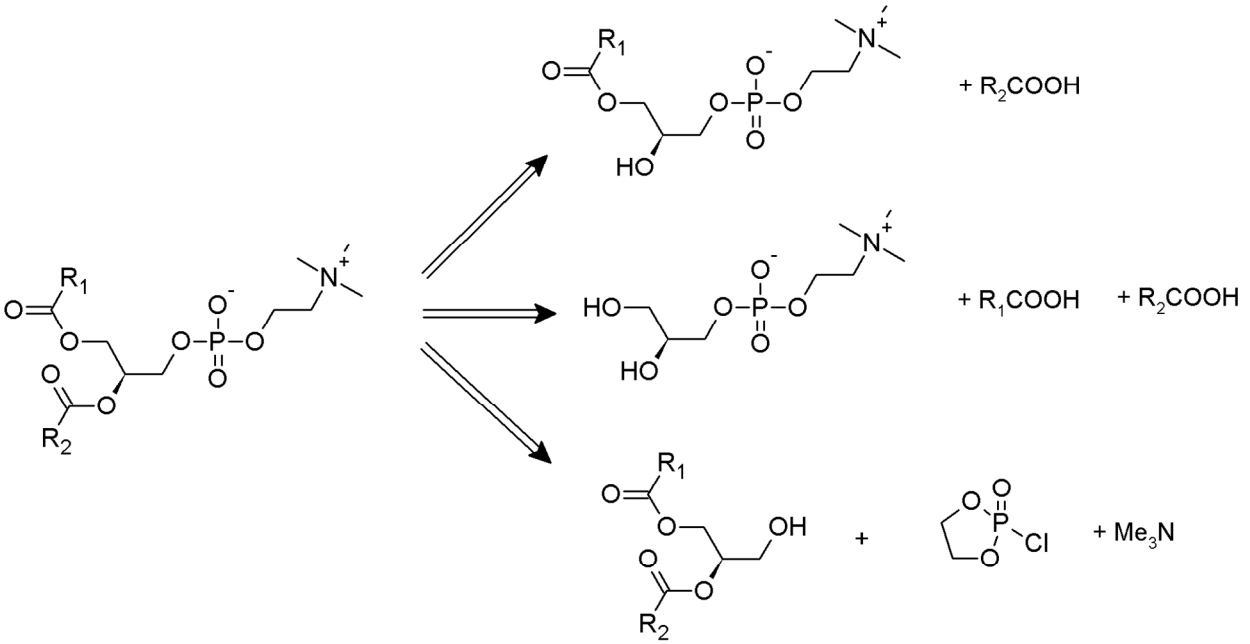
Phosphatidylcholine (PC) derivatives have been widely used to study bilayer properties,[1] protein-lipid adducts,[2] and PC interactions with aromatic amino acids,[3] to carry drug loads,[4] to identify,[5] monitor,[6] and inhibit[7] enzymes, to analyze enzyme mechanisms, to modulate cell activity,[9] to develop cosmetics[10] and coatings,[11] to enable imaging,[12] therapy,[13] and gene delivery,[14] and to perform total syntheses of natural products.[15] We are interested in using a PC derivative to study accumulation of drugs and drug-like compounds in the headgroup stratum of bilayers of biological membranes, and to analyze the influence of drug structure on the process.[16] Drug interactions with the headgroup and core strata are weak and difficult to measure because of the microscopic dimensions. To facilitate the measurements, an abstract 'split' of the bilayer into phases of homogeneous compositions, representing the headgroups and the core, helps design surrogate systems, which can be produced and used in macroscopic quantities, and analyzed using conventional analytical techniques.

Surrogate systems have been widely used to investigate drug transport and accumulation phenomena for more than a century. Several solvent systems have been used to mimic the bilayer, initially as a whole and later as individual bilayer strata. Interestingly, the well-established reference solvent, wet 1-octanol, was mostly used as a bilayer surrogate[17] but also supposed to represent the headgroups in some studies.[18] A PC derivative should be a superb surrogate for the headgroups because PC represents the predominant weight fraction of lipids in human bilayers: 42-49 % for skeletal muscle, heart, and liver, and 41% for brain.[19] The solvation properties of the bilayer core can be emulated by n-hexadecane[20] and other alkanes[21] or alkenes[22] because of similar densities and composition.[23]

Fluid PC bilayers are most frequently used in drug accumulation and transport experiments. Based on experimental[24] and computational[25] analyses, they contain 6-16 water molecules per

headgroup, depending on the fatty acid chains, pressure, and temperature. This means that a good headgroup surrogate is a PC derivative that has high aqueous solubility in molar range and forms homogeneous solutions even at high concentrations. Since commercially available di-n-propanoyl PC has the critical micellar concentration equal to 450 mM,[26] and the diformyl PC would provide additional H-bonding not found in PC,[27] diacetyl phosphatidyl choline (DAcPC) was an obvious candidate to examine.

Despite ample literature precedence for the synthesis of PC lipid derivatives, a reliable and scalable single-step process for the synthesis of DAcPC has not yet been identified. The reported procedures typically incorporate a hydrophobic carboxylic acid to facilitate the purification of the final compound. Syntheses of PC derivatives (Scheme 1) employed either L-α-glycerophosphocholine (GPC - middle), a monoester derivative of GPC (top) or a diester derivative of glycerol, into which the PC moiety was incorporated (bottom).



Scheme 1. Retrosynthetic analysis of PC derivatives. The R_1 and R_2 substituents may be identical.

The PC derivatives (Scheme 1) containing hydrophobic fragments could be purified by column chromatography and similar techniques. Typically, the compounds were prepared in small quantities - milligrams to few grams per reaction. However, for large scale synthesis (100 g to 1 kg), these purification approaches may not be ideal.

For example, D'Arrigo et al[28] reported a procedure to make mixed short/long chain PCs in a two-step process. GPC was first converted to cyclic stannylene derivatives, followed by the treatment with the first acyl chloride to obtain the lyso-phospholipid, which was further acylated with acid anhydride. The process typically uses a long chain fatty acid to facilitate purification of the product among other synthetic goals. Similarly, Rosseto et al[29] performed a multistep synthesis of mixed PCs in which the polar phosphocholine group was incorporated in the last step. However, the final product purification likewise seemed to be facilitated by the hydrophobic carboxylic esters. This was also the case for the synthesis protocol of Roodsari et al[30] in which the PC was incorporated as the last step.

Basheer et al described an enzymatic production of phospholipids in a patent filing[31] but the process was optimized for fatty acids. Park et al[32] reported a multi-step synthetic procedure for DAcPC starting from glycidol that was converted into 1-*O*-benzyl ether derivative of glycerol. The hydroxyl groups at C-2 and C-3 of glycerol were then acetylated. The diacetylated product was converted into DAcPC in three steps, and DAcPC was purified by column chromatography. However, since we needed to produce DAcPC in large quantities (preferably larger than 100 gram batches), this procedure[32] didn't seem feasible as we had observed severe tailing of DAcPC during column purification (see below).

Puricelli[33] prepared DAcPC in two steps. First, 2-chloro-2-oxa-1,3,2-dioxaphospholane was condensed with L-1,2-di-*O*-acetylglycerol in ether in presence of triethylamine at room

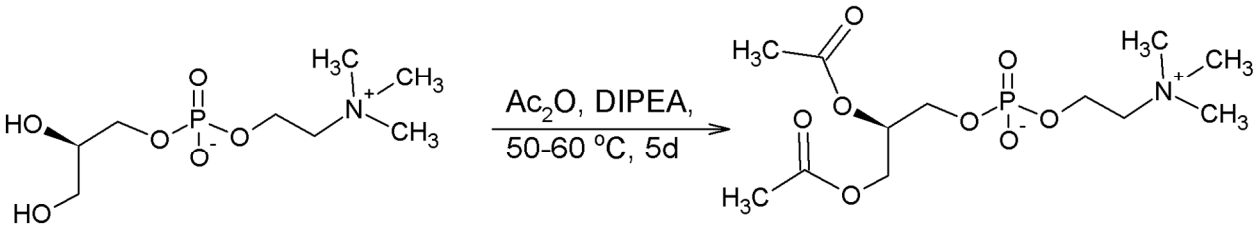
temperature. Then the condensate was reacted with excess trimethylamine under pressure at 1.5 atmospheres and 50 ºC to obtain DAcPC. The crude product was purified in a multistep process that involved filtering over carbon, ion exchange, vacuum concentration and recrystallization from acetone/alcohol mixtures. Alternatively, the crude DAcPC was subjected to column chromatography, a carbon treatment to remove color, and recrystallization from hydrated alcohol mixture (3% water in methanol). However, the author[33] did not describe the synthesis of α-1,2-di-*O*-acetylglycerol or its source. A literature search shows the racemic compound to be commercially available in gram quantities and its synthesis most likely involves a multistep process as described by Park et al.[32]

At the first attempt, we modified a procedure reported by Ichihara et al[34] to prepare DAcPC at a small scale. In the modified procedure, GPC adsorbed on to kieselguhr was suspended in dry chloroform and treated with excess acetic anhydride (2.5 molar equivalents) in presence of *N*,*N*-dimethylaminopyridine (DMAP) for 60 h. Although the procedure showed complete consumption of GPC, this reaction cannot be easily scaled up. For example, a gram-scale reaction needed more than a liter of methanol to wash DAcPC off the kieselguhr. A second challenge in the process was to isolate DAcPC from acetic acid byproduct and DMAP as the product seemed to have severe tailing when column chromatography was attempted with even 100% methanol containing 5% ammonia (~7 L solvent required to recover ~650mg DAcPC by normal column chromatography).

To satisfy the needs of researchers using DAcPC in their studies, including our laboratory, we developed a single step, scalable synthesis of DAcPC from commercially available compounds and purification of the product by distillation, crystallization, and washing. The details are summarized in this report.

Results and Discussion

Synthesis. The one-pot, scalable DAcPC synthesis using commercially available GPC, acetic anhydride (Ac_2O), and *N*,*N*-diisopropylethylamine (DIPEA) was performed at 50-60 °C for 5 days (Scheme 2). The used GPC was enantiopure, as confirmed by specific optical rotation, $[\alpha]_D^{25}$ = - 2.6 (*c* 2.7 in water), which is in good agreement with published data.[35]



Scheme 2. DAcPC synthesis from GPC using Ac_2O and DIPEA.

In a typical reaction, about 100 g of GPC was vacuum dried in a 500 mL Teflon® oven-dried flask, Ac_2O and DIPEA were added (4 molar and 3 molar equivalents, resp.), and the mixture was stirred at 55 °C for 5 days. The viscous GPC layer, adhering to the flask, gradually vanished, indicating the completion level of the reaction. Simultaneously, the mixture separated into two phases: a brown bottom layer containing most of the product, and a clear, almost colorless upper layer containing most of ammonium acetate. After disappearance of the GPC layer, the reaction mixture was stirred for additional 24 h.

Purification. Upon reaction completion (4-6 days), excess reagents and byproducts were stripped off under high vacuum at 110 °C (6 h) and 120 °C (2 h) in a glass round-bottom flask with constant stirring. The resulting reddish brown waxy solid was dissolved in 2 molar equivalents of water by stirring in a rotary evaporator at 80 °C without any vacuum. The resulting homogenous dark reddish brown solution was transferred into a 1 L Erlenmeyer flask, mixed with 1 L of acetone, and set for crystallization overnight at room temperature. DAcPC

crystallizes out as yellowish monohydrate (as inferred from ^{1}H NMR spectra – see below) in 50-80% yield in the first crop (m.p. = 96-98 ºC). The purity of the initial product was characterized by HPLC as 94 – 95 % (peak area ratio).

Apparently, a significant amount of impurities adhered to the surface of the crystals because they could be removed by washing by acetone or hexane with a weight loss 2-4 %, resulting in whitening of the crystals. The dried product was dissolved in a minimum amount of water and recrystallized from acetone, with 90 – 94 % yield. Repeated washing and crystallization provided better than 99 % purity, as determined by HPLC. For experiments using aqueous solutions, the impurities could also be extracted by partitioning into alkanes, while DAcPC remained in the aqueous solution. The impurities showed UV absorbance between 250 and 280 nm, DAcPC absorbs below 250 nm. The ^{1}H, ^{13}C, and ATR-IR spectra correspond with the DAcPC structure and are in good agreement with previously reported data.[32] The ^{1}H NMR spectra and peak assignments for lyophilized DAcPC are in Supporting Information.

Water Content. The exact water content of crystalline DAcPC is important for preparing the accurately defined aqueous solutions, serving as surrogates of bilayer headgroups under different conditions (temperature, fatty acid chains of PC, admixtures). DAcPC crystallizes as a monohydrate, as shown by a comparison of ^{1}H NMR spectra of purified DAcPC in $CDCl_3$ before and after lyophilization, which removes the water completely. If crystallized DAcPC monohydrate is left standing for days or weeks without desiccation, the water content increases to almost two molecules of water per DAcPC, although the crystals do not change visibly in the process. For such crystals, thermogravimetric (TGA) analysis in the nitrogen atmosphere showed that the major drop in weight occurred between 77.64 ºC and 117.47 ºC. This weight loss, attributed to the loss of water from the crystals, was 7.9 %, which was more than what was

expected for a monohydrate (5.0 %). The sample was decomposed at about 220 ºC. The Karl Fisher titration of the air-exposed DAcPC crystals showed the water content of 9.3 %, almost double that of monohydrate.

Crystallinity. The X-ray diffraction analysis showed four peaks with the relative intensity of more than 90 % and numerous smaller peaks. The peaks were not analyzed in detail but their presence confirmed the crystalline nature of isolated DAcPC solids. The DSC analysis of air-exposed crystals showed two asymmetric endothermic peaks. The larger peak at 96.97 ºC (melting point) started at about 50 ºC, pointing to the possibility of gradual loss of water. The smaller peak at 142.82 ºC could indicate a crystal form transition.

Enantiomer Analysis. Aqueous solutions of DAcPC exhibit specific optical rotation $[\alpha]_D^{25}$ = +6.51 (*c* 3.4 in water). This value is in good agreement with the data for 1,2-diacyl-3-*sn*-phosphatidyl cholines with different fatty acids, having $[\alpha]_D^{25}$ values from +5.10 to +7.02 in methanol/chloroform mixtures.[30,36] The published[32] value $[\alpha]_D^{25} = +12.11$ (*c* 1.8 in methanol) for DAcPC seems to be a bit out of this range. To analyze whether DAcPC is a mixture of enantiomers, a HPLC analysis with a chiral column was used. An isocratic protocol using the ChiralPak IC-3 column with ethanol as eluent did not show the presence of two enantiomers. These data indicate that synthesized and purified DAcPC is enantiopure.

EXPERIMENTAL SECTION

Materials. L-α-glycerophosphocholine (GPC) was procured from Waterstone Technologies, Carmel, IN in 2.5 kg batches. Acetic anhydride (Ac_2O) and *N*,*N*-diisopropylethylamine (DIPEA) were purchased from Alfa Aesar in liter quantities, and used as received.

1,2-Diacetyl-3-*sn*-Phosphatidyl Choline. About 100 g of GPC was placed in a 500 mL round-bottom, oven-dried Teflon® flask. GPC was then dried under high vacuum created by an Edwards model RV8 pump (2.0 x 10-3 mbar ultimate total pressure, as per company specifications) over phosphorus pentoxide for at least 5 hrs. About 4 molar equivalents of Ac2O and 3 molar equivalents of DIPEA were added to GPC. The molar equivalence was calculated based on the weight of GPC after vacuum drying. The reaction mixture was stirred at 55 ºC for 4-6 days using an in-house built overhead stirrer. At reaction completion, the viscous GPC was no longer seen adhering to the flask, and the reaction mixture was biphasic, with a reddish brown bottom layer and a clear, faintly yellow to colorless, upper layer. The reaction mixture was transferred into a glass round-bottom flask and distilled 6 h under high vacuum created by an Edwards model RV8 pump at 110 ºC with constant stirring provided by a magnetic stir bar. The temperature was raised briefly (~2 h) to 120 ºC to distill off the DIPEA acetate salt to obtain a reddish brown waxy solid. The solid was dissolved in 2 molar equivalents of water by stirring the distillation flask in a rotary evaporator at 80 ºC without any vacuum. Once a homogenous solution was obtained, the dark reddish brown solution was transferred into a 1 L Erlenmeyer flask. About 1 L of acetone was added to this solution, and set for crystallization overnight at room temperature. DAcPC crystallizes out as monohydrate with 50-80% yield in the first crop having 94 – 95 % purity (HPLC peak area ratio).

Washing with acetone or hexane resulted in whitening of the crystals, with a weight loss of 2-4 %. Crude DAcPC (30 g) was placed in a 500 mL Erlenmeyer flask and dissolved, with sonication, in minimum quantity of water (about 9 mL). Acetone (500 mL) was added to the flask and the solution was set for recrystallization (48-72 h) in the hood at room temperature. The solid DAcPC was collected by decantation and washed thrice with 60 mL of acetone each

time. The recrystallized DAcPC was dried in an oven at 37 ºC for 24-48 h. The yield was 90-94 %. The recrystallization process was repeated in case any impurity peak was observed in the UV or ^{1}H NMR spectra.

The product was characterized as follows: m.p. = 96-98 ºC; ^{1}H NMR ($CDCl_3$, 500 MHz, Brucker): after synthesis - δ 2.04 (s, 3H), 2.06 (s, 3H), 3.35 (s, 9H), 3.79 (m, 2H), 3.93 (m, 2H), 4.13 (dd, *J* =6.5, 12.0 Hz, 1H), 4.20 (m, 2H), 4.27 (m, 2H – water), 4.35 (dd, *J* = 3.0, 12.0 Hz, 1H), 5.17 (m, 1H); after lyophilization (see Supplementary Information) - δ 2.00 (s, 3H), 2.02 (s, 3H), 3.34 (s, 9H), 3.78 (m, 2H), 3.90 (m, 2H), 4.10 (dd, *J* = 6.9, 12.1 Hz, 1H), 4.25 (m, 2H), 4.31 (dd, *J* = 3.3, 12.1 Hz, 1H), 5.13 (m, 1H); ^{13}C NMR: (CD_3OD, 125 MHz, Bruker) δ 20.6, 20.9, 54.7, 60.5, 63.7, 64.8, 67.5, 72.0, 172.1, 172.4 ppm; MS: (ESI, Agilent 6130 single quadrupole) molecular ion calculated m/z 341.2946 (M), found 341.3 (M) and 364.1 ($M + Na^{+}$); ATR-IR (Alpha-E, Bruker) CH3 (s) 2960.44, CH_2 (s) and CH (s) 2892.01, CH_2 (b) and CH (b) 1485.89 and 1364.30, C=O (s) 1720.86, C-O-C (s) 1049.00, OH (s) in water 3391.61, P=O (s) 1380.49, P-O-C (s) 972.06 cm^{-1}; XRD (PANalytical X'Pert Pro, CuKa radiation, 40 kV, 40mA) 6.764, 15.394, 16.892, 20.195 °2Th.

HPLC Analyses. Two protocols were used: one with the main aim to determine the purity of the product and the other for characterizing enantiomer composition. The analyses were run on Waters e2695 HPLC with 2998 PDA detector. The DAcPC sample concentrations were 10-20 mg/mL, volume 10-20 μL, and temperature 25 ºC. The analyses lasted 15-25 min and the DAcPC elution time was 11-12 min.

The purity determination procedure used 250 mm × 4.6 mm, 3.5 μm, C18 Waters XBridge column. The protocol was extensively optimized to ensure that: (1) all peaks are separated; (2) the tailing factor of the DAcPC peak is minimal (<2.0); (3) the USP plate count for the DAcPC

peak is more than 10,000; and (4) the RSD of the DAcPC peak area for five replicate injections is less than 2 %. The Limit of Detection and the Limit of Quantitation were determined by the noise ratio method as 7.5 ppm and 20 ppm. The linearity was established for the DAcPC concentrations between 20 ppm and 3000 ppm. The procedure can be used to assess the purity of prepared DAcPC based on the ratio of the DAcPC area and total area. Two mobile phases were used: Milli-Q produced water with pH adjusted to 2.5 with ortho phosphoric acid (A) and acetonitrile (B). The gradient program was optimized as follows (time in min: % A, % B): 0: 99, 1; 3.5: 97, 3; 5.5: 97, 3; 6.5: 94, 6; 10.0: 94, 6; 12.0: 99, 1; 15.0: 99, 1; with the flow rate 1.0 mL/min.

The enantiomer characterization procedure was run in isocratic mode. The ChiralPak IC-3 (250 mm x 4.6 mm), 3μm column and ethanol as eluent were used.

Attenuated Total Reflectance Infrared Analysis. A sample of crystallized DAcPC (10.0 mg) was placed on the ATR crystal and scanned for the frequencies from 4000 cm^{-1} to 650 cm^{-1} (Alpha–E with software OPUS version 7.0, Bruker). The frequencies are listed as stretching (s) or bending (b).

X-Ray Diffraction. The crystalline DAcPC sample (500 mg) was used to make the pure material pellet, which was analyzed in a wide angle X-ray diffractometer (X'Pert Pro, PANanalytical). The peaks with the relative intensity above 90 % are listed. Many more peaks were observed.

Thermogravimetry. The crystalline DAcPC (9.712 mg) in a platinum crucible pan was placed into a TA Q-500 TGA analyzer, equilibrated at 30 ºC, and heated at 10 ºC/min to 250 ºC. The weight was taken in 0.5 sec intervals. The flow of nitrogen over the sample was 60 mL/min and over the balance 40 mL/min.

Differential Scanning Calorimetry. A pan was filled with the crystalline DAcPC (5.10 mg), lidded, and pressed using the sample encapsulating press. A crimped empty sample pan and lid were used as a reference. The sample was placed in the TA Q-2000 DS calorimeter with the nitrogen flow 50 mL/min and was equilibrated at 30 ºC. The temperature was raised to 250 ºC and then decreased back to 30 ºC, both at 10 ºC/min. The heat flow was measured in 0.5 sec intervals.

Karl Fisher Titration was used to determine the water content of air-exposed crystals. The DAcPC (about 100 mg) was transferred to the vessel of the autotitrator (Metrohm Titranido-905 with Ti stand 803, software Tiamo 2.4). The titration was run in duplicate.

Polarimetry. Two aqueous concentrations of DAcPC (34 and 102 g/L) were analyzed using Advanced Research Instruments PA-IR polarimeter at room temperature. The results showed small nonlinearity in the dependence of specific optical rotation on concentration (the $[\alpha]_D^{25}$ values +6.51 and +6.02 for the lower and higher concentration, respectively), so the value for the lower concentration was reported.

AUTHOR INFORMATION

Corresponding Author

*E-mail: stefan.balaz@acphs.edu

Notes. The authors declare no competing financial interest.

ACKNOWLEDGEMENTS

The authors thank to Dr. Senthil Natesan for fruitful discussions leading to the expansion of the palette of characterization methods. The research was in part supported by the NIH grant GM80508.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

^{1}H NMR spectrum of lyophilized 1,2-diacetyl-3-*sn*-phosphatidyl choline in $CDCl_3$ with peak assignment.

Reference List

1. (a) Cui, J.; Lethu, S.; Yasuda, T.; Matsuoka, S.; Matsumori, N.; Sato, F.; Murata, M. *Bioorg. Med. Chem. Lett.* **2015**, *25, 203-206*. (b) Gagnon, M. C.; Turgeon, B.; Savoie, J. D.; Parent, J. F.; Auger, M.; Paquin, J. F. *Org. Biomol. Chem.* **2014**, *12, 5126-5135*. (c) Krause, M. R.; Daly, T. A.; Almeida, P. F.; Regen, S. L. *Langmuir* **2014**, *30, 3285-3289*. (d) Yasuda, T.; Kinoshita, M.; Murata, M.; Matsumori, N. *Biophys. J.* **2014**, *106, 631-638*.

2. (a) Gubbens, J.; Ruijter, E.; de Fays, L. E., V; Damen, J. M.; de Kruijff, B.; Slijper, M.; Rijkers Dirk, T. S.; Liskamp Rob, M. J.; de Kroon, K. A., *Chem. Biol.* **2009**, *16, 3-14*. (b)

Tallman, K. A.; Kim, H. Y.; Ji, J. X.; Szapacs, M. E.; Yin, H.; McIntosh, T. J.; Liebler, D. C.; Porter, N. A. *Chem. Res. Toxicol.* **2007**, *20, 227-234*.

3. (a) Sanderson, J. M.; Whean, E. J. *Phys. Chem. Chem. Phys.* **2004**, *6, 1012-1017*. (b) Sanderson, J. M. *Org. Biomol. Chem.* **2007**, 5, 3276-3286.

4. (a) Li, Q.; Tian, Y.; Li, D.; Sun, J.; Shi, D.; Fang, L.; Gao, Y.; Liu, H. *Biomaterials* **2014**, *35, 6462-6472*. (b) Bayer, A. M.; Alam, S.; Mattern-Schain, S. I.; Best, M. D. *Chem. - Eur. J.* **2014**, *20, 3350-3357*.

5. Long, J. Z.; Cisar, J. S.; Milliken, D.; Niessen, S.; Wang, C.; Trauger, S. A.; Siuzdak, G.; and Cravatt, B. F. *Nat. Chem. Biol.* **2011**, *7, 763-765*.

6. (a) Popov, A. V.; Mawn, T. M.; Kim, S.; Zheng, G.; Delikatny, E. J. *Bioconjugate Chem.* **2010**, *21, 1724-1727*. (b) El Alaoui, M.; Noiriel, A.; Soulere, L.; Grand, L.; Queneau, Y.; Abousalham, A. *Anal. Chem.* **2014**, *86, 10576-10583*.

7. Falconi, M.; Ciccone, S.; D'Arrigo, P.; Viani, F.; Sorge, R.; Novelli, G.; Patrizi, P.; Desideri, A.; Biocca, S. *Biochem. Biophys. Res. Commun.* **2013**, *438, 340-345*.

8. Liu, W.; Porter, N. A.; Schneider, C.; Brash, A. R.; Yin, H. *Free Radical Biol. Med.* **2011**, *50, 166-178*.

9. Egger, J.; Bretscher, P.; Freigang, S.; Kopf, M.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2013**, *52, 5382-5385*.

10. Pifferi, G.; Anzaghi, P.; Stefli, R. 2002, EP2641[2002072591]

11. Dietz, U.; Nubbemeyer, U. Germany. 2012, EP60773[2012168342].

12. Ng, K. K.; Shakiba, M.; Huynh, E.; Weersink, R. A.; Roxin, A.; Wilson, B. C.; Zheng, G. *ACS Nano* **2014**, *8, 8363-8373*.

13. He, C.; Liu, D.; Lin, W. *ACS Nano* **2015**, *9, 991-1003*.

14. Prata, C. A. H.; Li, Y.; Luo, D.; McIntosh, T. J.; Barthelemy, P.; Grinstaff, M. W. *Chem. Commun.* **2008**, *1566-1568*.

15. (a) Duclos, R. I., Jr. *Chem. Phys. Lipids* **2010**, *163, 102-109*. (b) Acharya, H. P.; Kobayashi, Y. *Angew. Chem., Int. Ed.* **2005**, *44, 3481-3484*.

16. (a) Natesan, S.; Lukacova, V.; Ming, P.; Subramaniam, R.; Lynch, S.; Wang, Z.; Tandlich, R.; Balaz, S. *Mol. Pharmaceutics* **2014**, *11, 3577-3595*. (b) Lukacova, V.; Natesan, S.; Peng, M.; Tandlich, R.; Wang, Z.; Lynch, S.; Subramaniam, R.; Balaz, S. *Mol. Pharmaceutics* **2013**, *10, 3684-3696*. (c) Lukacova, V.; Peng, M.; Tandlich, R.; Hinderliter, A.; Balaz, S. *Langmuir* **2006**, *22, 1869-1874*.

17. (a) Collander, R. *Acta Chem. Scand.* **1951**, *5, 774-780*. (b) Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86, 1616-1626*. (c) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71, 525-616*.

18. (a) Xiang, T. X.; Anderson, B. D. *J. Pharm. Sci.* **1994**, *83, 1511-1518*. (b) Wimley, W. C.; White, S. H. *Nat. Struct. Biol.* **1996**, *3, 842-848*.

19. *Geigy Scientific Tables*, 8th ed.; Ciba-Geigy Corporation: West Caldwell, NJ, 1986; pp 1-300.

20. Scheuplein, R. J.; Blank, I. H.; Brauner, G. J.; MacFarlane, D. J. *J. Invest. Dermatol.* **1969**, *52, 63-70*.

21. Chikhale, E. G.; Ng, K. Y.; Burton, P. S.; Borchardt, R. T. *Pharm. Res.* **1994**, *11, 412-419*.

22. Xiang, T. X.; Anderson, B. D. *J. Membr. Biol.* **1994**, *140, 111-122*.

23. Marrink, S. J.; Berendsen, H. J. C. J. Phys. Chem. **1994**, *98, 4155-4168*.

24. (a) Balgavy, P.; Dubnickova, M.; Kucerka, N.; Kiselev, M. A.; Yaradaikin, S. P.; Uhrikova, D. *Biochim. Biophys. Acta* **2001**, *1512, 40-52*. (b) Barry, J. A.; Gawrisch, K. *Biochemistry* **1994**, *33, 8082-8088*. (c) Hristova, K.; White, S. H. *Biophys. J.* **1998**, *74, 2419-2433*.

25. Tieleman, D. P.; Marrink, S. J.; Berendsen, H. J. *Biochim. Biophys. Acta* **1997**, 1331, 235-270.

26. Martin, S. F. and Pitzer, G. E. *BBA Biomembr.* **2000**, *1464, 104-112*.

27. Desiraju, G. R. *Acc. Chem. Res.* **1996**, *29, 441-449*.

28. D'Arrigo, P.; Fasoli, E.; Pedrocchi-Fantoni, G.; Rossi, C.; Saraceno, C.; Tessaro, D.; Servi, S. *Chem. Phys. Lipids* **2007**, *147, 113-118*.

29. Rosseto, R.; Bibak, N.; Hajdu, J. *Org. Biomol. Chem.* **2006**, *4, 2358-2360*.

30. Roodsari, F. S.; Wu, D.; Pum, G. S.; Hajdu, J. *J. Org. Chem.* **1999**, *64, 7727-7737*.

31. Basheer, S.; Zuabi, R.; Shulman, A.; Mar-Chaim, N. 2002-IL344[2002090560].

32. Park, J. M.; De Castro, K. A.; Ahn, H.; Rhee, H. *Bull. Korean Chem. Soc.* **2010**, *31, 2689-2691*.

33. Puricelli, L. 1992, EP-102731[502357].

34. Ichihara, K.; Iwasaki, H.; Ueda, K.; Takizawa, R.; Naito, H.; Tomosugi, M. *Chem. Phys. Lipids* **2005**, *137, 94-99*.

35. Milne, G. W. A. *Drugs: Synonyms and Properties*, Ashgate Publishing Company, Brookfield, VT, 2000; p. 1280. (b) Baer, E.; Kates, M. *J. Am. Chem. Soc.* **1948**, *70*, 1394-1399.

36. (a) Baer, E.; Buchnea, D.; Newcombe, A. E. *J. Am. Chem. Soc.* **1956**, *78*, 232-257. (b) Bibak, N.; Hajdu, J. *Tetrahedron Lett.* **2003**, *44*, 5875-5877. (c) Baer, E.; Kates, M. *J. Am. Chem. Soc.* **1950**, *72*, 942-949. (d) Hanahan, D. J. *J. Biol. Chem*. **1954**, *211*, 321-325.