HOKKAIDO UNIVERSITY

Title	Rapid tin-mediated access to a lysophosphatidylethanolamine (LPE) library: Application to positional LC/MS analysis for hepatic LPEs in non-alcoholic steatohepatitis model mice
Author(s)	Furukawa, Takayuki; Fuda, Hirotoshi; Miyanaga, Satoshi et al.
Citation	Chemistry and Physics of Lipids, 200, 133-138 https://doi.org/10.1016/j.chemphyslip.2016.09.003
Issue Date	2016-10-01
Doc URL	https://hdl.handle.net/2115/67258
Rights	©2016, Elsevier. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	Chem Phys Lipids_200_133-138.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Rapid tin-mediated access to a lysophosphatidylethanolamine (LPE) library: application to positional LC/MS analysis for hepatic LPEs in non-alcoholic steatohepatitis model mice

Takayuki Furukawa, Hirotoshi Fuda, Satoshi Miyanaga, Chinatsu Watanabe, Hitoshi Chiba, and Shu-Ping Hui*

Graduate School of Health Science, Hokkaido University, North 12, West 5, Kita-ku, Sapporo, 060-0812 Japan.

Email: keino@hs.hokudai.ac.jp, Phone: +81-11-706-3693

Abstract. Even though lysophospholipids have attracted much interest in recent years on account of their unique bioactivity, research related to lysophospholipids is usually hampered by problems associated with standard sample preparation and discrimination of regioisomers. Herein, we demonstrate a quick tin-chemistry-based synthetic route to lysophosphatidylethanolamines (LPEs) and its application in the positional analysis of hepatic LPEs in non-alcoholic steatohepatitis (NASH) model mice. We found that the preference of hepatic LPE regioisomer largely depends on the unsaturation of acyl chain in both control and NASH model mice. In addition, hepatic C18:2-LPE and C20:5-LPE levels were significantly lower in the NASH model mice than those in the control. The LC/MS technique based on the library of LPE regioisomers allows an accurate observation of hepatic LPE metabolism and might provide useful information to elucidate yet ambiguous pathogenesis of NASH.

Keywords. Lysophosphatidylethanolamine, Tin, Regioisomer, non-alcoholic steatohepatitis, LC/MS

Abbreviations. Tr: triphenylmethyl, DPPE: 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, PE: phosphatidylethanolamine, LPL: lysophospholipid, LPE: lysophosphatidylethanolamine, LPC: lysophosphatidylcholine, LPI: lysophosphatidylinositol, LPA: lysophosphatidic acid, LPS: lysophosphatidylserine, PL: phospholipid, HG: head group, iPrOH: isopropanol, DMF: dimethylformamide, Bu_2SnO: dibutyltinoxide, Teoc: 2-(trimethylsilyl)ethoxycarbonyl, TFA: trifluoroacetic acid, FA: fatty acid, PUFA: polyunsaturated fatty acid, NASH: non-alcoholic steatohepatitis, NAFLD: non-alcoholic fatty liver disease

1. Introduction.

Phospholipids (PLs) are fundamental biomolecules that consist of a polar head group, a glycerol backbone, and fatty acids. Their importance in membrane assembly and molecular signaling as e.g. lipid mediators has been well documented (Aoki, et al., 2015). Lysophospholipids (LPLs) represent a PL subclass and contain only one fatty acid on the glycerol backbone. Recent studies revealed that these unique PLs, especially lysophosphatidic acids (LPAs), contribute to an array of biological responses such as platelet aggregation, smooth muscle contraction, cell proliferation, and cell migration (Mills and Moolenaar, 2003) *via* binding to the corresponding receptors. Compared to LPAs or lysophosphatidylserines (LPSs) (Makide, et al., 2014) the functions of lysophosphatidylethanolamines (LPEs) still remain undiscovered, which is mostly due to the limited commercial availability of LPEs.

Another frequently encountered problem in LPE and LPL research is 1,2-*O*-acyl migration (Figure 1) (Plückthun and Dennis, 1982; Liu, et al., 2014), as this equilibrium furnishes 1-LPE and 2-LPE regioisomers, which exhibit different physico-chemical (Okudaira, et al., 2014) and biological (Xu, et al., 2005) properties. Unfortunately, suppression of this migration still remains difficult, and accordingly the precise determination of the regioisomer ratio prior to a use of the mixture is of paramount importance. NMR spectroscopy can be a powerful tool for this purpose (Medina and Sacchi, 1994), even though it requires relatively large sample quantities and is thus not ideally suited for the analysis of biological samples. Conversely, mass spectrometry (MS) is more advantageous in terms of its detection limit, and has already been applied to the characterization of various LPLs (Hsu and Turk, 2009). Nevertheless, their complete identification still requires the use of standard samples.

Herein, we report a new short synthetic route to LPEs, which is suitable for the construction of an LPE library. Moreover, we applied this synthetic library into the positional analysis of LPEs in

murine liver tissues by using LC-MS platform. In this study, we employed a non-alcoholic steatohepatitis (NASH) model mice developed before (Yimin, et al., 2012), and compared the hepatic levels of LPE regioisomers between the NASH model and the control mice. NASH is a progressive hepatic disease developed in association with fatty liver and multiple risk factors such as oxidative stress and proinflammatory factors. The pathogenesis of NASH is yet ambiguous, and therefore, no specific diagnosis/treatment/prevention method is available. This study aims to obtain useful information for understanding of the pathogenic processes of NASH from the viewpoint of hepatic LPE metabolism.

2. Experimental

2.1. Materials and methods

Unless otherwise noted, all reagents were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan) or Sigma-Aldrich. TLC was performed on pre-coated plates (20 cm × 20 cm; layer thickness: 0.25 mm; silica gel $60F_{254}$; Merck), and spots were visualized by spraying with an ethanol solution of Ninhydrin, followed by heating to 250 °C for ~ 0.5 min or by exposure to UV light (λ = 254 nm) when applicable. Column chromatography on silica gel (N60; spherical type; particle size: 40-50 μm; Kanto Chemical Industry) was carried out using the specified solvent systems, whereby the solvent ratio is given in v/v. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz JNM-ECP400 spectrometer (JEOL, Japan; ^{1}H: 400 MHz, ^{13}C: 100 MHz), and multiplicities are given as singlet (s), broad (br), doublet (d), doublet of doublets (dd), triplet of doublets (td), triplet (t), quintet (q), or multiplet (m). Chemical shifts are expressed in ppm and referenced to TMS (δ_H 0.00), $CHCl_3$ (δ_H 7.26), CH_3OD (δ_H 3.31), $CDCl_3$ (δ_C 77.16), or CD_3OD (δ_C 49.00) as the internal standard (Fulmer, et al., 2010). Assignments in the ^{1}H NMR spectra were made by first-order analysis using the ACD/NMR processing software (Advanced Chemistry Development, Inc.). High-resolution electrospray ionization mass spectra (HR-ESI-MS) and liquid chromatography mass spectra (LC-MS) were recorded on an LTQ Orbitrap XL (Thermo Fisher Scientific), equipped with a Shimadzu Prominence HPLC system.

2.2 Liver tissues and lipid extraction

Murine liver tissues were prepared as we reported previously (Yimin, et al., 2012). Briefly, NASH model mice were produced by the combination of high-fat diet for 23 weeks and several intravenous injections of oxidized low-density lipoproteins. This model shows histhopathological

and metabolic features similar to human NASH (Yimin, et al., 2012). Mice fed a regular diet were used for control study. Hepatic lipids were extracted from a liver tissue (~50 mg wet weight) using a conventional Folch method (Folch, et al., 1957). Animal experiments were conducted according to the Regulations for the Care and Use of Laboratory Animals of Hokkaido University. Our experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Hokkaido University (Approval No. 10-0028)

2.3. LC-ESI-MS/MS conditions and procedures

LPE regioisomers were separated on an HPLC with an Atlantis T3 column (3 μm x 2.1 mm x 150 mm; Waters) using an aqueous AcOH (0.1%) / acetonitrile gradient system (flow rate: 0.3 mL min^{-1}; for details, see: SI). The following ESI conditions were applied: capillary voltage = 4000 V, desolvation temperature = 350 °C, sheath gas = 50 psi, and auxiliary gas = 10 psi. LPE precursor ions and product ions were detected in positive ion mode; for LPE precursor ions: FTMS (resolution: 60000); for product ions: ITMS (CID normalized collision energy = 35 V). Obtained data were analyzed using the Qual browser (Thermo Fisher Scientific). 10 μL of hepatic lipid extracts was injected, and extracted ion chromatogram (EIC) was obtained on the basis of theoretical molecular weight (10 ppm error range) for each LPEs. Regioisomer ratios were calculated based on integrated peak areas. All assays were done in five replications: livers from five different NASH model and control mice were analyzed.

2.4. Data processing

Relative intensity was calculated by integrated peak area over liver weight. Statistical differences were analyzed by two-tailed Mann-Whitney U test (Prism 6, GraphPad Software Inc.) and a value of $P < 0.05$ was regarded as significant.

3. Results and discussion

3.1. Chemical synthesis of an LPE library

Although the chemical synthesis of several LPEs has already been reported (D'Arrigo and Servi, 2010), most of these examples represent a 'total synthesis' approach. Even though such a strategy is undoubtedly useful, it is not suitable for the construction of a library, as it is simply too time consuming. We envisaged that a semi-synthetic approach should be more favorable for the construction of such a library, as it may potentially require significantly less steps. It should also be noted here that the chemical semi-synthesis of LPEs still remains unexplored.

Our "semi-synthesis" strategy is shown in Figure 2. As a starting point, we chose commercially available diacyl-phosphoethanolamine **1** (PE). The protection of the amino functionality and a subsequent deacylation should afford intermediate **2**. A regioselective mono-acylation of this diol **2** with a variety of fatty acid derivatives, followed by the deprotection of the amino group should furnish the targeted LPE molecules in only two steps.

For the protection of the amino functionality in commercially available 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE, **4**), we chose the trityl group, which have already successfully employed in a previous PE synthesis (Aneja, et al., 1969; Plückthun, et al., 1985). The protection of the amino group in **4** using tritylbromide and triethylamine proceeded quantitatively, and the subsequent deacylation under Zemplén conditions (Zemplén and Pacsu, 1929) afforded the desired amino-protected diol **6** in 87% yield (Scheme 1).

With ready access to intermediate **6**, we proceeded to the subsequent acylation step. Given that the target of this synthetic route was to synthesize LPE in a highly regioselective manner, we attempted a tin-mediated activation, given the proven track record of this method in carbohydrate chemistry (Jäger and Minnaard, 2015). Moreover, this approach has already been successfully applied to the synthesis of LPCs by Servi *et al*. (Fasoli, et al., 2006). When we applied this

approach to diol **6**, we obtained trityl-protected LPEs **7'-17'** in yields that were merely moderate (18-44%) compared to previously reported LPCs (Scheme 2) (Fasoli, et al., 2006; Niezgoda, et al., 2013). The results of these experiments are summarized in Table 1. During these experiments, we realized that this acylation step is very sensitive to water. When solvents were not dried enough prior to use, decreased yields were observed (entries 4 and 7, Table 1). A solvent screening revealed that DMF afforded more reproducibility than conventional isopropanol (data not shown). Even though trityl-protected LPEs **7'-17'** were soluble in $CDCl_3$, we were unable to obtain useful NMR spectra, and observed only broad features. Therefore, we proceeded to the final deprotection step without prior NMR analysis. A subsequent work-up under acidic conditions afforded LPEs **7-17** in good yield (35-80%) (Table 1). After deprotection, we observed regioselectivity ratios (2-LPE:1-LPE) of 3:1 to 7:3, with the exception of 3:2 for **15** (entry 9, Table 1). Even though these ratios are not as high as for the previously reported LPCs (Fasoli, et al., 2006; Niezgoda, et al., 2013), they are nevertheless acceptable, considering the aforementioned problematic acyl migration. Even if the acylation was highly regioselective, the acidic work-up should promote a 1,2-*O*-acyl migration and thus lower the selectivity. It is generally considered that the regioselectivity of tin-mediated reaction is induced by steric effect: More sterically favorable *sn*-1 OH reacts prior to more hindered *sn*-2 OH (Roelens, 1996).

We also tested the Teoc protecting group in order to shield the amino functionality (Scheme 3), as Teoc can be removed under milder conditions than the trityl group. Fortunately, this change increased the yield in the acylation step to 72%. Unfortunately, the deprotection was not successful at all. A fluoride-induced removal did not proceed, and treatment with TFA afforded complex product mixtures. This result indicates that the judicious choice of protecting group for the amino functionality should be critical for both acylation and deprotection.

3.2. Analysis of LPE regioisomers by LC/MS

So far, discrimination of regioisomers has been carried out predominantly by two methods: NMR spectroscopy and mass spectrometry. While NMR is able to provide a variety of information, it requires relatively high sample quantities, and its applicability thus depends on the solubility of the LPE, which in turn largely depends on the fatty acid moiety. LPEs with PUFA chains tend to exhibit higher solubility than those with saturated FA chains. For instance, LPE **17** with a DHA FA chain is soluble in methanol, while LPE **9** with a stearic acid chain is virtually insoluble, even in chloroform/methanol or dimethylsulfoxide (DMSO). This difference restricts the versatility of the NMR approach in our study. Conversely, mass spectrometry requires very small sample quantities, which renders solubility virtually irrelevant. Especially liquid chromatography tandem-mass spectrometry (LC-MS/MS) represents a powerful approach to distinguish 1/2-LPEs (Han and Gross, 1996; Fang, et al., 2003; Lee, et al., 2011).

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in worldwide and might progress into non-alcoholic steatohepatitis (NASH) known as more severe state of disease (Musso, et al., 2016). Many studies have investigated the lipid content in a liver tissue of NASH or NAFLD (Byeon, et al., 2016; García-Cañaveras, et al., 2011; Eisinger, et al., 2014; Gorden, et al., 2015; Nam, et al., 2015). Most of the previous studies on hepatic fat focused on triglycerides (TG) metabolism, because TG is the major constituent of hepatic fat. On the other hand, no statement concerning LPE regioisomers can be found in the literature to our best knowledge.

In order to understand pathogenic processes in NASH, we established a novel NASH murine model and characterized by morphological, histological and biochemical means (Yimin, et al., 2012). This model was confirmed to have increased TG, free fatty acid, and total cholesterol in the liver. However, no information about other class of lipids in this model has been available so

far. Therefore, we performed further analysis of hepatic lipids in this model, especially focused on LPEs, using LC/MS and a synthetic library as references.

At first, we established an LC/MS condition to separate LPEs and regioisomers (see SI). So as to characterize LPEs in LC-MS, we measured retention times (Table 2) and confirmed a fragmentation pattern in MS/MS spectra. Although three olefinic isomers of C18:3 (**12**-**14**) could not be separable in this condition, other LPEs were possible. Regioisomers can be distinguished by the LC and MS/MS because similar fragmentation patterns are observed as reported (Fang, et al., 2003; Lee, et al., 2011). Briefly, 2-LPE series provided in general highly intense $[M+H-H_2O]^+$ signals and moderately intense $[M+H-HG]^+$. In contrast, the 1-LPE series exhibited an opposite fragmentation pattern, i.e. strong $[M+H-HG]^+$ and very weak $[M+H-H_2O]^+$ signals (Figure 3 and SI).

With diagnostic tools in hand, we began to analyze hepatic LPEs using the same LC-MS condition. Results are summarized in Table 2. Surprisingly, the positional preferences in hepatic LPEs are largely dependent on the degree of unsaturation of acyl chain (Figure 4); C16:0 (**8**) and C18:0 (**9**) LPEs are found in exclusively 2- LPE form, however, C20:4 (**15**), C20:5 (**16**), and C22:6 (**17**) LPEs are observed in exclusively 1-LPE form. C18:1 (**10**) and C18:2 (**11**) LPEs appear transitional; C18:1 (**10**) is 2-LPE rich whereas C18:2 (**11**) is 1-LPE rich. This remarkable difference can't be explained by the theory of chemical equilibrium: Thermodynamically more stable 2-LPE (1-Acyl) should be favorable than 1-LPE (2-Acyl) as reported in previous studies (Plückthun and Dennis, 1982; Liu, et al., 2014). Thus, a contribution of biological or enzymatic factors is anticipated: for example, the different substrate specificity of lipases in a liver tissue, such as LPA1 or LPA2 (Okudaira, et al., 2014), but this hypothesis needs to be proven in future. It is also noteworthy that there is no clear difference between control and NASH in this point.

Conversely, significant differences are observed in relative abundance (Figure 5). In C18:2 (**10**) and C20:5 (**16**) LPEs, decreased intensities are detected in a NASH liver tissue. Although other LPE species are not significant, diminishing trend is found in whole series with the exception of C20:4 (**15**). As similar trend was documented in the analysis of human liver tissues (Gorden, et al., 2015), our murine model showed closer outcome in human, so we consider that our NASH model mice would be valuable to understand pathogenic processes of NASH in human.

4. Conclusion

Despite the growing interest on LPLs as lipid mediators, synthetic access and appropriate characterization tools still remain limited. In this study, we focused on LPEs, whose functions are not yet well understood. We developed a short novel method for the synthesis of various LPEs, and applied into the analysis of hepatic LPE extracted from NASH model and control mice.

We showed that the diol skeleton can be successfully modulated by a tin-mediated acylation that has previously been employed successfully in carbohydrate chemistry. This synthetic route to LPEs requires in total only four steps and is thus substantially less laborious than conventional routes. Using this route, we constructed an LPE library including eleven FAs. We also found that this tin-mediated acylation is highly sensitive to water and that the selection of the protecting group for the amino moiety should be important for further improvements. These results should also be helpful for the synthesis of other LPE analogues or other classes of LPLs.

In the LC/MS study, we demonstrated that synthetic LPEs and its regioisomers exhibit distinct retention times with an exception of C18:3 series and confirmed that regioisomers can be discriminated by a fragmentation pattern in MS/MS. In the analysis of hepatic LPEs, we found that the positional preference largely depends upon the degree of unsaturation of acyl chain. Strikingly highly-unsaturated LPEs (**15**-**17**) were found in exclusively 1-LPE form, which should be thermodynamically less stable. Moreover, there are significant differences in the relative abundance of C18:2 (**11**) and C20:5 (**16**) LPEs between NASH and control murine liver tissues. These findings strengthen the importance of standard compounds, and thus we believe that our new synthetic method can contribute to further development of LPE research.

Acknowledgement. This study was financially supported by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) in the context of the Regional Innovation Strategy Support Program Sapporo Health Innovation "Smart-H".

Appendix A. Supplementary data

Experimental details and identification data are included in the supporting information, which can be found at Chemistry and Physics of Lipids website (http://www.sciencedirect.com/science/journal/00093084)

References

Aneja, R., Chadha, J., Davies, A., Rose, C., 1969. The condensation of aziridine with phosphatidic acid; synthesis of O-(1,2-diacyl-sn-glycero-3-phosphoryl)-ethanolamine. Chem Phys Lipids, 3, 286–291. DOI: 10.1016/0009-3084(69)90020-6

Aoki, J., Kawana, H., Kise, R., 2015. Second generation of lipid mediators: lysophospholipid Exp Med, 33, 2349-2360

Byeon, S. K., Lee, J. C., Chung, B. C., Seo, H. S., Moon, M. H., 2016. High-throughput and rapid quantification of lipids by nanoflow UPLC-ESI-MS/MS: application to the hepatic lipids of rabbits with nonalcoholic fatty liver disease. Anal Bioanal Chem, 408, 4975-4985. DOI: 10.1007/s00216-016-9592-y

D'Arrigo, P., Servi, S., 2010. Synthesis of lysophospholipids. Molecules, 15, 1354-1377. DOI: 10.3390/molecules15031354

Eisinger, K., Krautbauer, S., Hebel, T., Schmitz, G., Aslanidis, C., Liebisch, G., Buechler, C., 2014. Lipidomic analysis of the liver from high-fat diet induced obese mice identifies changes in multiple lipid classes. Exp Mol Pathol, 97, 37-43. DOI: 10.1016/j.yexmp.2014.05.002

Fang, N., Yu, S., Badger, T., 2003. LC-MS/MS analysis of lysophospholipids associated with soy protein isolate. J Agr Food Chem, 51, 6676-6682. DOI: 10.1021/jf034793v

Fasoli, E., Arnone, A., Caligiuri, A., D'Arrigo, P., Ferra, L., Servi, S., 2006. Tin-mediated synthesis of lyso-phospholipids. Org Biomol Chem, 4, 2974-2978. DOI: 10.1039/B604636C

Folch, J., Lees, M., Sloane-Stanley, G. H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem, 226, 497-509

Fulmer, G., Miller, A., Sherden, N., Gottlieb, H., Nudelman, A., Stoltz, B., Bercaw, J., Goldberg, K., 2010. NMR chemical shifts of trace impurities: common laboratory solvents, organics, and gases in deuterated solvents relevant to the organometallic chemist. Organometallics, 29, 2176-2179. DOI: 10.1021/om100106e

García-Cañaveras, J. C., Donato, M. T., Castell, J. V., Lahoz, A., 2011. A Comprehensive Untargeted Metabonomic Analysis of Human Steatotic Liver Tissue by RP and HILIC Chromatography Coupled to Mass Spectrometry Reveals Important Metabolic Alterations J Proteome Res, 10, 4825-4834. DOI: 10.1021/pr200629p

Gorden, D., Myers, D., Ivanova, P., Fahy, E., Maurya, M., Gupta, S., Min, J., Spann, N., McDonald, J., Kelly, S., Duan, J., Sullards, M., Leiker, T., Barkley, R., Quehenberger, O., Armando, A., Milne, S., Mathews, T., Armstrong, M., Li, C., Melvin, W., Clements, R., Washington, M., Mendonsa, A., Witztum, J., Guan, Z., Glass, C., Murphy, R., Dennis, E., Merrill, A., Russell, D., Subramaniam, S., Brown, H., 2015. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. J Lipid Res, 56, 722–736. DOI: 10.1194/jlr.P056002

Han, X., Gross, R., 1996. Structural determination of lysophospholipid regioisomers by electrospray ionization tandem mass spectrometry. J Am Chem Soc, 118, 451-457. DOI: 10.1021/ja952326r

Hsu, F.-F., Turk, J., 2009. Electrospray ionization with low-energy collisionally activated dissociation tandem mass spectrometry of glycerophospholipids: Mechanisms of fragmentation and structural characterization. J Chromatogr B, 877, 2673-2695. DOI: 10.1016/j.jchromb.2009.02.033

Jäger, M., Minnaard, A., 2015. Regioselective modification of unprotected glycosides. Chem Commun, 52, 656-664. DOI: 10.1039/C5CC08199H

Lee, J., Min, H., Moon, M., 2011. Simultaneous profiling of lysophospholipids and phospholipids from human plasma by nanoflow liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem, 400, 2953–2961. DOI: 10.1007/s00216-011-4958-7

Liu, L., Tong, C., Bao, J., Waters, D., Rose, T., King, G., 2014. Determination of starch lysophospholipids in rice using liquid chromatography–mass spectrometry (LC-MS). J Agr Food Chem, 62, 6600-6607. DOI: 10.1021/jf500585j

Makide, K., Uwamizu, A., Shinjo, Y., Ishiguro, J., Okutani, M., Inoue, A., Aoki, J., 2014. Novel lysophosphoplipid receptors: their structure and function. J Lipid Res, 55, 1986–1995. DOI: 10.1194/jlr.R046920

Medina, I., Sacchi, R., 1994. Acyl stereospecific analysis of tuna phospholipids via high resolution 13C-NMR spectroscopy. Chem Phys Lipids, 70, 53–61. DOI: 10.1016/0009-3084(94)90047-7

Mills, G.B., Moolenaar, W.H., 2003. The emerging role of lysophosphatidic acid in cancer. Nat. Rev. Cancer, 3, 582-591. DOI: 10.1038/nrc1143

Musso, G., Cassader, M., Gambino, R., 2016. Non-alcoholic steatohepatitis: emerging molecular targets and therapeutic strategies. Nat. Rev. Drug Discovery, 15, 249-274. DOI:10.1038/nrd.2015.3

Nam, M., Choi, M.-S., Jung, S., Jung, Y., Choi, J.-Y., Ryu, D., Hwang, G.-S., 2015. Lipidomic Profiling of Liver Tissue from Obesity-Prone and Obesity-Resistant Mice Fed a High Fat Diet Sci Reports, 5, 16984. DOI: 10.1038/srep16984

Niezgoda, N, Mituła, P, Kempińska, K, Wietrzyk, J., 2013. Synthesis of phosphatidylcholine with conjugated linoleic acid and studies on its cytotoxic activity. Aust J Chem, 66, 354-361. DOI: 10.1071/CH12404

Okudaira, M., Inoue, A., Shuto, A., Nakanaga, K., Kano, K., Makide, K., Saigusa, D., Tomioka, Y., and Aoki, J., 2014. Separation and quantification of 2-acyl-1-lysophospholipids and 1-acyl-2-lysophospholipids in biological samples by LC-MS/MS. J Lipid Res, 55, 2178-2192. DOI: 10.1194/jlr.d048439

Plückthun, A., Dennis, E.A., 1982. Acyl and phosphoryl migration in lysophospholipids: importance in phospholipid synthesis and phospholipase specificity. Biochemistry, 21, 1743-1750. DOI**:** 10.1021/bi00537a007

Plückthun, A., Rohlfs, R., Davidson, F. F., Dennis, E. A., 1985. Short-chain phosphatidylethanolamines: physical properties and susceptibility of the monomers to phospholipase A2 action. Biochemistry, 24, 4201-4208. DOI: 10.1021/bi00336a058

Roelens, S., 1996. Organotin-Mediated Monoacylation of Diols with Reversed Chemoselectivity. Mechanism and Selectivity. J Org Chem, 61, 5257-5263. DOI: 10.1021/jo960453f

Xu, Y., Aoki, J., Shimizu, K., Umezu-Goto, M., Hama, K., Takanezawa, Y., Yu, S., Mills, G., Arai, H., Qian, L., and Prestwich, G., 2005. Structure-activity relationships of fluorinated lysophosphatidic acid analogues. J Med Chem, 48, 3319-3327. DOI: 10.1021/jm049186t

Yimin, Furumaki, H., Matsuoka, S., Sakurai, T., Kohanawa, M., Zhao, S., Kuge, Y., Tamaki, N., Chiba, H., 2011. A novel murine model for non-alcoholic steatohepatitis developed by

combination of a high-fat diet and oxidized low-density lipoprotein. Lab invest, 92, 265-281. DOI: 10.1038/labinvest.2011.159

Zemplén, G., Pacsu, E., 1929. Über die verseifung acetylierter zucker und verwandter substanzen. Ber Dtsch Chem Ges, 62, 1613-1614.

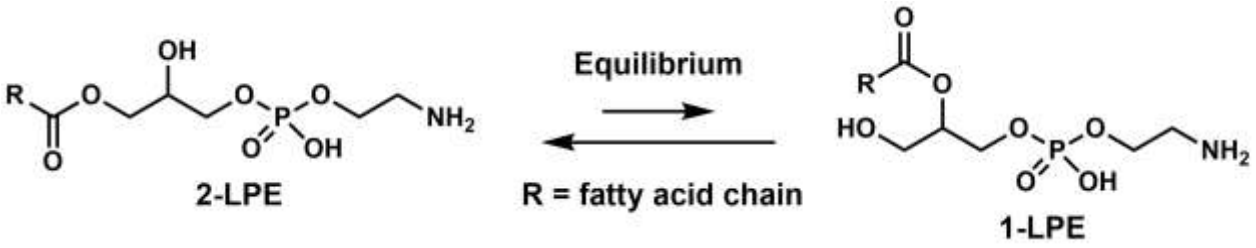


Figure 1. 1,2-*O*-acyl migration in LPEs

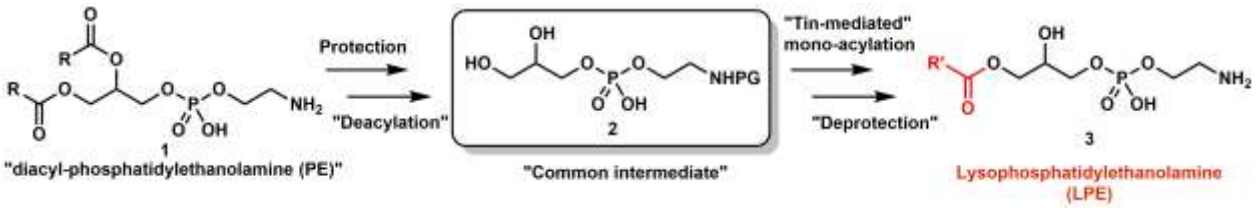


Figure 2. Synthetic strategy towards LPEs in this study; R, R'= fatty acid chains ; PG = protecting group

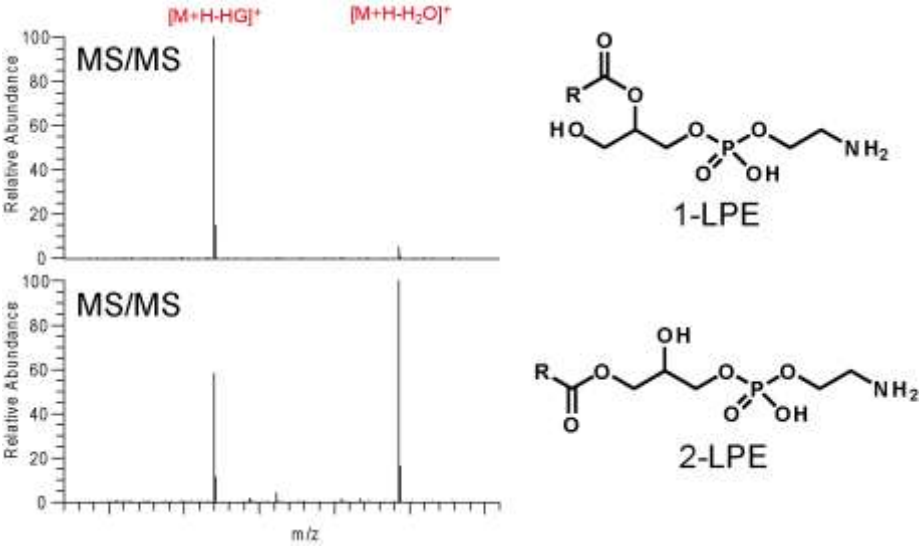


Figure 3. Representative product ion pattern for 1- (top) and 2-LPEs (bottom).

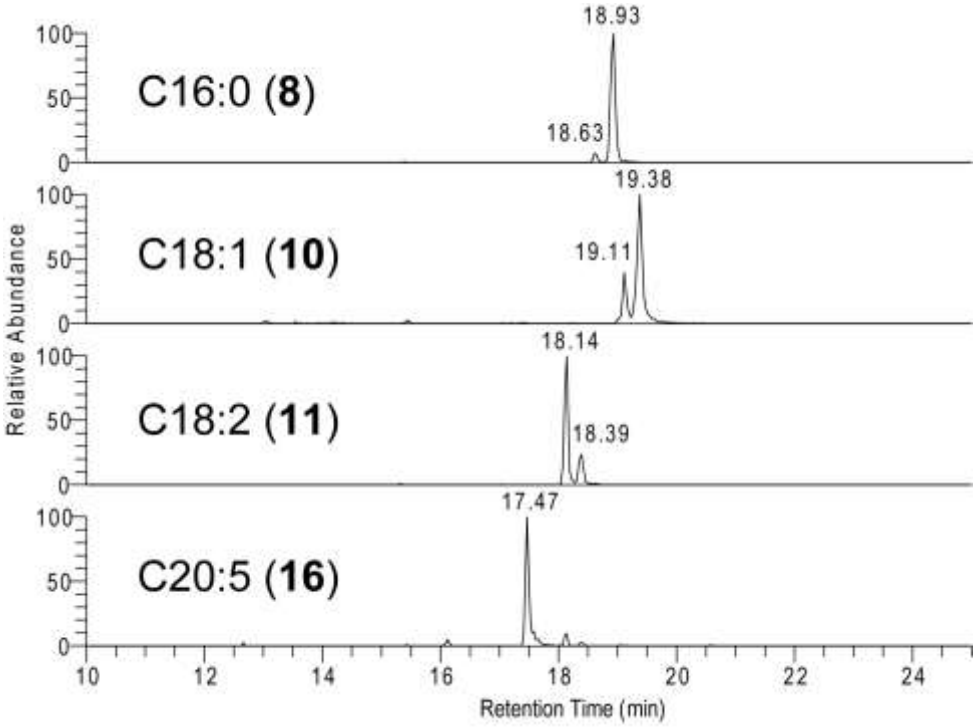


Figure 4. Positional preferences of C16:0 (**8**), C18:1 (**10**), C18:2 (**11**) and C20:5 (**16**)

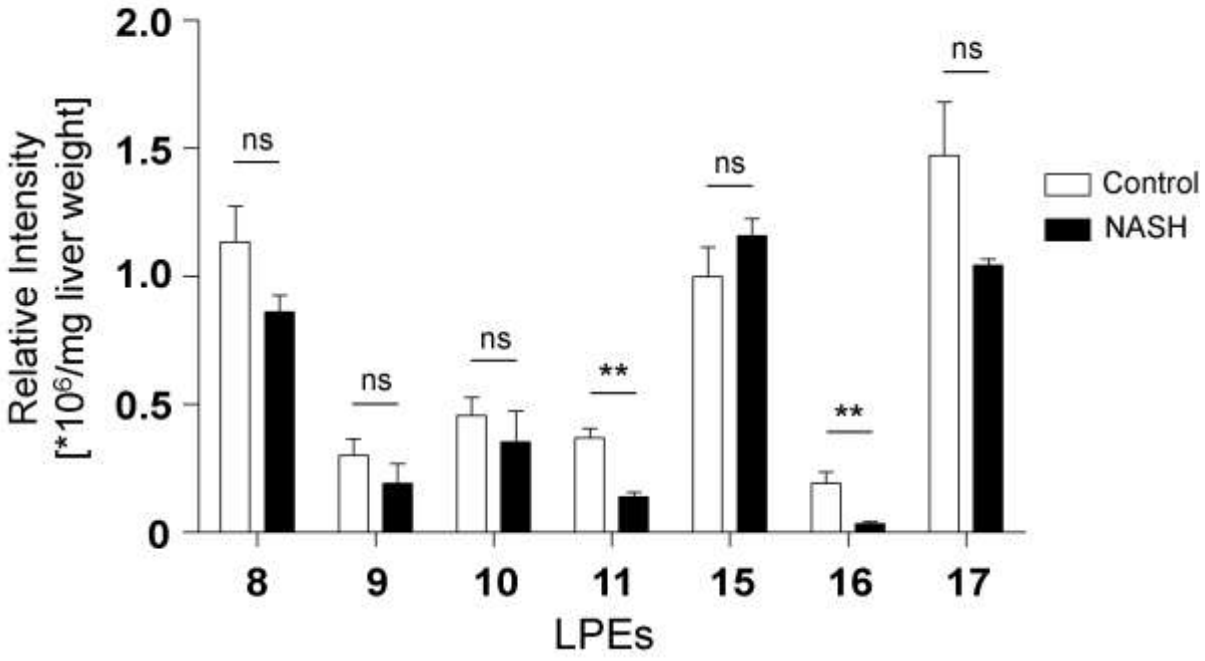
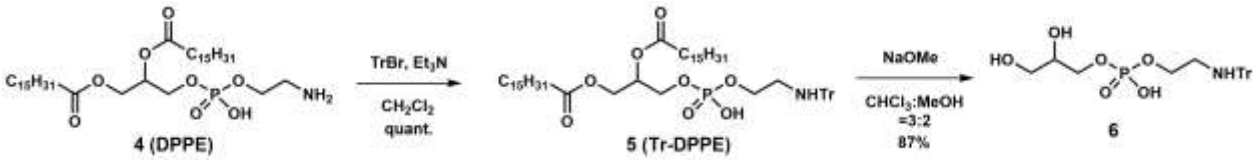
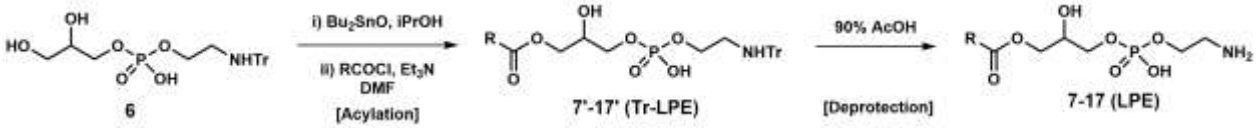


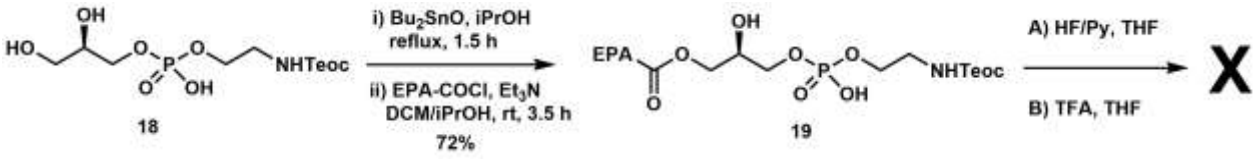
Figure 5. Comparison of NASH and control liver tissues. C18:3 (**12-14**) were excluded for clarity. N = 5, ns: not significant, **: $P < 0.01$



Scheme 1. Synthesis of common intermediate **6**



Scheme 2. Tin-mediated acylation of **6** and subsequent deprotection of **7**'-**17**'



Scheme 3. Protecting the amino functionality with a Teoc group

Entry	RCOCl (R =)	Yield (%)		2-LPE:1-LPE
		Acylation	Deprotection	
1	C10:0 (capric acid) **7**	27	54	3:1
2	C16:0 (palmitic acid) **8**	18	77	7:3
3	C18:0 (stearic acid) **9**	18	69	7:3
4	C18:1 (oleic acid) **10**	11	56	7:3
5	C18:2 (linoleic acid) **11**	44	60	3:1
6	C18:3 (pinolenic acid) **12**	26	47	3:1
7	C18:3 (α-linolenoic acid) **13**	10	59	3:1
8	C18:3 (γ-linolenoic acid) **14**	26	69	3:1
9	C20:4 (arachidonic acid) **15**	27	37	3:2
10	C20:5 (EPA) **16**	37	79	7:3
11	C22:6 (DHA) **17**	38	80	3:1

Table 1. Product yields for each step and selectivity values for the regioisomers

LPE	Retention Time (min)			
	Synthetic Reference		Control	NASH
	1-LPE	2-LPE		
C10:0 (**7**)	14.48	14.73	N.D.	N.D.
C16:0 (**8**)	18.65	18.98	18.63, 18.93†	18.64, 18.95†
C18:0 (**9**)	20.29	20.69	20.64†	20.69†
C18:1 (**10**)	19.11	19.40	19.11, 19.38†	19.12, 19.41†
C18:2 (**11**)	18.16	18.42	18.14†, 18.39	18.14†, 18.39
C18:3 (**12-14**)*	17.4-17.8		17.4-17.8	17.4-17.8
C20:4 (**15**)	18.23	18.44	18.21†, 18.43	18.23†, 18.42
C20:5 (**16**)	17.49	17.69	17.47†	17.48†
C22:6 (**17**)	18.22	18.41	18.18†, 18.37	18.18†, 18.36

Table 2. LC-MS analysis of liver LPEs from control and NASH model mice. *: detected as a overlapped signal, † : observed as a main peak and confirmed by MS/MS, N.D. : not detected