A simple and efficient method for synthesis of Sn-Glycero-phosphoethanolamine

Siddabasave Gowda B. Gowda[1], Hirotoshi Fuda[1], Yusuke Yamamoto[2], Hitoshi Chiba[3], and Shu-Ping Hui[1*]

1. Faculty of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan
2. Graduate School of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan
3. Department of Nutrition, Sapporo University of Health Sciences, Nakanuma, Nishi 4-2-1-15, Higashi-ku, Sapporo 007-0894, Japan

* Correspondence: Shu-Ping Hui.

Faculty of Health Sciences, Hokkaido University,

Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan.

E-mail: keino@hs.hokudai.ac.jp,

Tel. & Fax: +81-11-706-3693.

Abstract:

An efficient three-step strategy for the convenient synthesis of *Sn*-glycero-3-phosphoethanolamine (GroPEtn) from a commercially available 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) is reported. Direct hydrolysis of DPPE produce a complex inseparable mixture, hence a protection and deprotection strategy is employed to prepare GroPEtn. The primary amine of DPPE is protected with a highly stable acid-labile trityl group, followed by strong base hydrolysis of *N*-trityl-DPPE gives *N*-trityl-GroPEtn. Further a mild, rapid, and efficient deprotection method is established using trifluoroacetic acid to remove N-trityl moiety, affords GroPEtn as a single product. This is the first semisynthetic approach and efficient method to produce GroPEtn with a total yield of 66% in three steps. GroPEtn did not show any cytotoxicity against human kidney (HK-2) cells and reporter gene assay for activation of Keap1-Nrf2 mediated antioxidant defense mechanism showed no significant effects.

Keywords: Glycerophospholipids, Sn-Glycero-phosphoethanolamine**,** acid hydrolysis**,** trityl deprotection, Nuclear-magnetic resonance, High-resolution mass spectrometry.

Abbreviations and Acronyms

GPL: Glycerophospholipids

GroPEtn: *Sn*-Glycero-3-phosphoethanolamine

DPPE: 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine

HK-2: Human kidney-2

NMR: Nuclear-magnetic resonance

HRMS: High-resolution mass spectrometry

Introduction

Glycerophospholipids (GPL) are the major components of cell membranes that have important structural and functional properties(Casares *et al.* 2019). Chemically, they are the derivatives of sn-glycero-3-phosphoric acid, synthesized by a de novo pathway at endoplasmic reticulum and are transported to other membranous structures by phospholipid exchange and transfer proteins(Fagone and Jackowski 2009). Several types of GPL are synthesized with a varying polar head at the sn-3 position of the glycerol backbone, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylglycerol (PG). In addition to forming the physical boundary of cells with a suitable environment, fluidity, and ion-permeability, GPL are involved in fundamental regulatory cellular functions such as cell signalling and as an anchor for proteins in cell membranes(Frisardi *et al.* 2011). Phosphatidylethanolamine (PE) is one of the two-major GPL (along with PC) found in cell membranes and is known to inhibit the mitochondrial respiratory activity and reduced in age-related hippocampal neuron death in rodent models(Modica-Napolitano and Renshaw 2004).

Sn-Glycero-3-phosphoethanolamine (GroPEtn) is a breakdown product of PE, and present in higher levels in normal liver relative to other organs(Tallan *et al.* 1954). It is known to stimulate the growth of hepatocytes and dropped significantly during liver regeneration(Nelson *et al.* 1996; Houweling *et al.* 1992). GroPEtn showed the enhanced activity of epidermal growth factor in cultured hepatocytes(Nelson *et al.* 1996). Even though several methods for synthesis of PE and lyso-PE were reported (Rakhit *et al.* 1969; Furukawa *et al.* 2016; D'Arrigo and Servi 2010) there are no reports on the efficient synthesis of GroPEtn. An effort to prepare GroPEtn starting from a glycerol derivative has been reported, however, it is limited by multi-step reactions and poor reaction yield (Baer and Stancer 1953). GroPEtn is also attempted to prepare by acidic hydrolysis of alkali stable phospholipid fraction extracted from brain, however the yield is low and complexity exist in purification process(Ansell and Spanner 1963). GroPEtn is a strong polar molecule with enhanced water solubility and as reactive free amine moiety, which often cause the reaction by-products during the course of synthesis. Due to growing interest in GroPEtn bioactivities, there is a crucial need for the efficient method to produce GroPEtn. Herein, we report a three-step semisynthetic approach for the synthesis of GroPEtn, which is suitable for the efficient production GroPEtn in high yield. Also, its cell cytotoxicity and the role in Keap1-Nrf2 (Kelch ECH associated protein 1-nuclear factor erythroid 2-related factor 2) activation is examined.

Materials and Method

Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, $CDCl_3$, trifluoroacetic acid, trityl bromide, and sodium methoxide were obtained from Tokyo Chemical Industry (Tokyo, Japan), CD_3OD is obtained from Cambridge Isotope Laboratories, Inc.,. All other reagents of synthetic grade and LC-MS grade (methanol) were purchased from Wako Pure Chemical Corporation (Tokyo, Japan). TLC was performed on Merck pre-coated plates (20 cm × 20 cm; layer thickness, 0.25 mm; Silica Gel $60F_{254}$); the spots were visualized by spraying Ninhydrin in ethanol or 5% H_2SO_4 in methanol when applicable. Silica Gel N60 (spherical type, particle size 40-50 μm; Kanto Chemical Industry) was used for column chromatographic purification. Proton and carbon NMR was recorded with 400 MHz JNM-ECX400P (JEOL, Japan; ^1H: 400 MHz, ^{13}C: 100 MHz); multiplicities are given as singlet (s), broad (br), doublet (d), double doublets (dd), triple doublets (td), triplet (t), quintet (q), or multiplet (m). Chemical shifts are given in ppm. ^1H NMR spectra were processed by ACD/NMR processor software (Advanced Chemistry Development, inc.). High-resolution electrospray ionization mass spectra (HR-ESI-MS) was recorded by Linear trap quadrupole (LTQ) Orbitrap XL (Thermo Fisher scientific). Low-resolution electrospray ionization mass spectra (LR-ESI-MS) was recorded by LXQ (Thermo Fisher scientific).

N-Trityl-1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (Tr-DPPE); To a solution of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine **1** (DPPE: 100 mg, 0.144 mmol) in dichloromethane (5 mL) and trimethylamine (97 μL, 4.8 eq, 0.693 mmol), Trityl bromide (84.26 mg, 1.8 eq, 0.2607 mmol) was added and stirred for overnight at room temperature under nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure and resulting mixture was purified by flash column chromatography ($CHCl_3$:MeOH = 1:0~20:1, by vol) to give Tr-DPPE **2** (122 mg, 91%) as a white solid. The ^1H-NMR and ^{13}C-NMR of the compound were identical to our previous report (Furukawa *et al.* 2016) (See Supporting Information). R_f = 0.42 ($CHCl_3$:MeOH = 7:1, by vol); ^1H-NMR (400 MHz, $CDCl_3$) δ 7.53-7.24 (m, 15 H), 5.14 (br m, 1 H), 4.32-4.29 (br d, 1 H, J = 11.3 Hz), 4.12-4.08 (br m, 2 H), 3.82 (br m, 2 H), 3.04 (br s, 1 H), 2.22-2.15 (br m, 4 H), 1.56 (br s, 4 H), 1.24 (br s, 48 H), 0.9-0.84 (t, 6 H, J = 6.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.30, 128.97, 128.87, 128.34, 128.07, 73.85, 63.89, 63.85, 62.64, 39.7, 34.21, 34.04, 31.89, 29.69, 29.63, 29.33, 29.13, 25.62, 24.83, 22.65, 14.07; HR-ESIMS calculated for $C_{56}H_{87}O_8NP$ $[M - H]^-$, 932.6175, found, 932.6165 (-1.07 ppm).

N-Trityl-1,2-dihydroxy-*sn*-glycero-3-phosphoethanolamine (Tr-GroPEtn): To a solution of Tr-DPPE **2** (120 mg, 0.128 mmol) in chloroform (3 mL) and methanol (2 mL), sodium methoxide (15.2 mg, 2.2 eq, 0.282 mmol) was added and stirred for 5 h at room temperature. The reaction mixture was concentrated under reduced pressure and resulting mixture was purified by flash column chromatography ($CHCl_3$:MeOH:H_2O = 9:1:0~65:25:4, by vol) to give Tr-GroPEtn **3** (53.2 mg, 90%) as a white solid. The ^1H-NMR and ^{13}C-NMR of the compound

were identical to our previous report (Furukawa *et al.* 2016) (See Supporting Information). R_f = 0.30 ($CHCl_3$:MeOH:H_2O = 65:25:4, by vol); ^{1}H-NMR (400 MHz, CD_3OD) *δ* 7.42-7.27 (m, 15 H), 4.08-3.94 (m, 2 H), 3.93-3.89 (m, 2 H), 3.87-3.75 (m, 1 H), 3.60-3.51 (m, 2 H), 3.27-3.25 (m, 1 H), 2.75 (br s, 2 H); ^{13}C-NMR (100 MHz, CD_3OD) *δ* 142.53, 142.50, 129.63, 129.31, 128.83, 128.71, 74.51, 72.13, 72.06, 67.68, 67.62, 63.49; HR-ESIMS calculated for $C_{24}H_{27}O_6NP$ $[M - H]^-$, 456.1581, found, 456.1582 (+2.2 ppm).

***Sn*-Glycero-3-phosphoethanolamine(GPEA):** N-Trityl-1,2-dihydroxy-*sn*-glycero-3-phospho ethanolamine (Tr-GroPEtn) **3** (50 mg, 0.109 mmol) was dissolved in 3 ml of anhydrous methylene chloride/trifluoroacetic acid, 2:1, by vol.). The yellow solution was stirred at 0 °C for 5 min and then rapidly neutralized with 6 ml of 14% aqueous ammonia. The reaction mixture was concentrated under reduced pressure and resulting residue is purified by column chromatography (elution starting with ethylacetate:methanol (4:1) to ethylacetate/methanol/water/acetic acid (2:1:1:0.3, by vol) to give GroPEtn **4** (19 mg, 81%) as a white solid. R_f = 0.26 (EtOAc:MeOH:H_2O:CH_3COOH = 2:1:1:0.3, by vol); ^{1}H-NMR (400 MHz, CD_3OD) *δ* 3.93 (m, 2 H), 3.64-3.78 (m, 2H), 3.46 (m, 1H), 3.64 (m, 1H), 3.16 (m, 1 H), 3.03 (m, 2H), 1.8 (brs, -NH_2); ^{13}C-NMR (100 MHz, CD_3OD) *δ* 72.65, 72.57, 68.02, 67.97, 63.90, 63.10, 61.62, 41.76, 41.70; HR-ESIMS calculated for $C_5H_{14}O_6NP$ $[M - H]^-$, 214.0487, found, 214.0488 (-0.46 ppm).

Cell cytotoxicity assay:

Normal human kidney-2 (HK-2, CRL-2190) cells (1 × 10^5/well) were seeded into 96-well plates with minimum essential medium (MEM, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin-Neomycin mixture (modified MEM). GroPEtn was pre-dissolved in MEM and applied to the cells and incubated at 37°C under a humidified atmosphere of 5% CO_2 in air for 24 h. Then the culture media was removed by vacuum suction and a freshly prepared 10 µL of CCK-8 reagent (Dojindo Molecular Technologies) and 200 µL of MEM were added, with additional incubation for about 1 h. Followed by 50 µL of 1% (w/v) sodium dodecyl sulfate solution was added to cease the reaction and the absorbance was recorded using Wallac 1420 ARVO Mx plate reader (PerkinElmer, Tokyo, Japan) at 450 nm. The half-maximal inhibitory concentration (IC_{50}) of GroPEtn was analyzed by Prism 6.03 software (GraphPad, San Diego, CA, USA).

Reporter gene assay for Keap1-Nrf2 signaling:

HK-2 cells (1 × 10^5/well) were seeded into 96-well plates with modified MEM and incubated at 37°C for 24 h. FuGENE HD Transfection Reagent was used with a reagent-to-total DNA ratio of 4:1 (µL/µg) to transfect the HK-2 cells according to manufacturer's protocol. The cells were co-transfected with two luciferase reporter vectors pGL4.37[luc2p/ARE/Hygro] and

pGL4.75[hRluc/CMV] at a 20:1 mass ratio. After 24 h of transfection, the transfection reagent was removed and GroPEtn was applied to the transfected cells and further incubated for 24 h. Then the luciferase activity was determined by Dual-Glo Luciferase Assay System (Promega) according to the manufacturer's protocol. Activity was measured with a Wallac 1420 ARVO Mx plate reader and corrected for transfection efficiency by normalizing to hRluc activity. Relative luciferase activity (fold) was calculated as the ratio of fluorescence intensity in GroPEtn treated samples to that in the untreated control sample.

Result and Discussion

Glycerophospholipids are the major membrane lipids with broad bioactivities and several synthetic routes were reported in the literature for its nonpolar derivatives (Ahmad *et al.* 2007; Leßig and Fuchs 2010). However, synthesis of more polar GPL was limited in the literature. In the course of our phospholipid research, we have developed a new and efficient route for the synthesis of GroPEtn. Despite the simple structure of GroPEtn there is no single efficient method of synthesis was reported. Due to the increasing biological importance of PE derived water-soluble metabolites such as GroPEtn, there is a need for an efficient method to prepare GroPEtn. To achieve this goal, we have designed a semisynthetic approach by using 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) as a starting material. At first, an effort to use direct alkali-mediated hydrolysis of DPPE as a strategy to obtain GroPEtn was carried out using strong bases such as sodium methoxide or aqueous potassium hydroxide. These attempts produced several inseparable by-products and in some cases ethanolamine as a major product rather the GroPEtn. Furthermore, an attempt using mild bases such as aqueous lithium hydroxide, potassium carbonate, and lithium carbonate were carried out but reaction does not proceed. All the experimental conditions attempted in this study were listed in **Table 1**. In our previous study involving synthesis of lyso-PE, we found that protection of amine group is necessary to carry out the direct alkali hydrolysis of DPPE (Furukawa *et al.* 2016). More specifically, trityl protection of primary amine of GroPEtn makes the N-trityl bond more stable during the synthesis and it is successfully employed in a previous PE and lyso-PE synthesis (Furukawa *et al.* 2016). A three-step semisynthetic approach for the synthesis of GroPEtn employed in the current study is shown in **Figure 1**.

The protection of an amine group of DPPE **1**, is carried out with tritylbromide to obtain N-trityl-DPPE **2**, and the subsequent deacylation using Zemplen conditions afforded the desired N-trityl-GroPEtn **3,** in 90% yield (Furukawa *et al.* 2016). The deacylation is carried out under alkali conditions using sodium methoxide (2.2 eq) in a slight excess amount to the previous method to accomplish the reaction towards completion and obtain a good yield. Next, most challenging task is the deprotection of the N-trityl moiety, which is often cleaved either by catalytic hydrogenation or by the treatment with acid. At first, the deprotection of trityl group

was carried out by the method reported earlier (Furukawa *et al.* 2016) in the synthesis of lyso-PE, using 90% acetic acid, refluxing at the 120 ^{o}C for 5 min. However, this deprotection approach produced several by-products introducing considerable difficulties in the final purification process. Use of strong acids or harsh reaction conditions produces complex mixture, due to the presence of reactive primary alcohol and ether moieties. In other words, mild conditions could favor the hydrolysis by suppressing reaction by-products.

In this work we designed and developed a very mild deprotection strategy using trifluoroacetic acid/dichloromethane (1:2) at 0 $^{\circ}$C for 5 min, which produced GroPEtn **4**, as a single product with a yield of 81%. The high yield of GroPEtn could be due to the complete progress of the reaction under mild condition in a short period of time without any by-products. Whereas direct hydrolysis of DPPE produces several by-products and often the product yield is very low or not obtained. The pictorial representation of the trityl deprotection reaction, thin-layer chromatographic detection, and spectra of high-resolution mass and MS/MS data of GroPEtn are shown in **Figure 2**. The detailed NMR assignment of GroPEtn is described in the method section and the spectra were provided in **Figure 3**. Unless stated harsh conditions or long reaction time produces a complex inseparable mixture which limits the supply of GroPEtn, whereas using this semisynthetic approach with facile acidic hydrolysis conditions we could overcome this issue and able explore the unknown biological functions of GroPEtn.

The Nrf2 is a transcription factor that regulates the expression of many phase II and antioxidant genes to maintain the cellular homeostasis. Small molecules are known to activate Nrf2 pathway and protect the cells from oxidative stress induced damages(Joko *et al.* 2017). Since, GroPEtn is a low molecular weight water soluble metabolite it is of great interest to explore its antioxidant property. At first, the cell cytotoxicity of the GroPEtn against human kidney-2 (HK-2) cells was tested using cell counting kit-8 (CCK-8) and the results are given in **Figure 4A**. The CCK-8 assay was carried out according to the previously published literature from our group(Joko *et al.* 2017). The data clearly show that GroPEtn as no toxicity even at higher concentrations such as 1000 μM, indicates its potential to use as pharmaceutical candidate where GroPEtn plays a beneficial role. Furthermore, to examine GroPEtn activity against Keap1-Nrf2 pathway, we tested its activity using HK-2 cells by reporter gene assay developed in our laboratory (Joko *et al.* 2017). The assay results showed that, GroPEtn as no significant role in the Keap-Nrf2 activation (**Figure 4B**), which is crucial in antioxidant defense mechanisms.

Conclusion

In this study, we successfully synthesized GroPEtn for the first time using a simple three-step approach with high efficiency in a short period of time. This strategy could avoid the severe

by-products produced by direct alkali hydrolysis and hence can be employed for the preparation of GroPEtn in high yield. Furthermore, GroPEtn did not show any toxicity against HK-2 cells and no significant role in Keap1-Nrf2 activation. Since bioactivities of GroPEtn is curbed by the lack of authentic standard, our study helps us to explore the importance of GroPEtn, a breakdown product of PE in many biological processes.

Conflict of Interest

The authors declare no conflict of interests

Acknowledgement

This study was financially supported by the Japanese Society for the Promotion of Science KAKENHI Grants (17H01879, 18K07434, 19H03117 and 19K07861). SGBG thanks faculty of health science for providing start-up grant (87011Q) and Mr Liang for technical support.

Supporting Information

The Supporting Information is available free of charge on the…

References

Ahmad M. U., Krishna U. M., Ali S. M., Choudhury S., Ahmad I. (2007) Synthesis of 1,3- and 1,2-diphosphatidylglycerol. *Lipids* **42**, 291–296. https://doi.org/10.1007/s11745-007-3020-1

Ansell G. B., Spanner S. (1963) The alkaline hydrolysis of the ethanolamine plasmalogen of brain tissue. *J. Neurochem.***10**, 941-945. https://doi.org/10.1111/j.1471-4159.1963.tb11921.x

Baer E., Stancer H. C. (1953) The Synthesis of O-(L-α-Glycerylphosphoryl)-ethanolamine and Some Comments on the Stereochemical Aspects of the Biosynthesis of Glycerolphosphatides from Carbohydrates. *J. Am. Chem. Soc.* **75**, 4510–4514. https://doi.org/10.1021/ja01114a035

Casares D., Escribá P. V., Rosselló C. A. (2019) Membrane lipid composition: Effect on membrane and organelle structure, function and compartmentalization and therapeutic avenues. *Int. J. Mol. Sci.* **20,** E2167. https://doi.org/10.3390/ijms20092167

D'Arrigo P., Servi S. (2010) Synthesis of lysophospholipids. *Molecules*.**15**, 1354-1377. https://doi.org/10.3390/molecules15031354

Fagone P., Jackowski S. (2009) Membrane phospholipid synthesis and endoplasmic reticulum function. *J. Lipid. Res.* **50**, S311-S316. https://doi.org/10.1194/jlr.R800049-JLR200

Frisardi V., Panza F., Seripa D., Farooqui T., Farooqui A. A. (2011) Glycerophospholipids and glycerophospholipid-derived lipid mediators: A complex meshwork in Alzheimer's disease pathology. *Prog. Lipid. Res*, **50**, 313-330. 10.1016/j.plipres.2011.06.001

Furukawa T., Fuda H., Miyanaga S., Watanabe C., Chiba H., Hui S. P. (2016) Rapid tin-mediated access to a lysophosphatidylethanolamine (LPE) library: Application to positional LC/MS analysis for hepatic LPEs in non-alcoholic steatohepatitis model mice. *Chem. Phys. Lipids* **200**, 133–138. https://doi.org/10.1016/j.chemphyslip.2016.09.003

Houweling M., Tijburg L. B. M., Vaartjes W. J., Golde L. M. G. Van (1992) Phosphatidylethanolamine metabolism in rat liver after partial hepatectomy. Control of

biosynthesis of phosphatidylethanolamine by the availability of ethanolamine. *Biochem. J.* **283**, 55–61. https://doi.org/10.1042/bj2830055

Joko S., Watanabe M., Fuda H., Takeda S., Furukawa T., Hui S. P., Shrestha R., Chiba H. (2017) Comparison of chemical structures and cytoprotection abilities between direct and indirect antioxidants. *J. Funct. Foods*. **20**, 516-531. https://doi.org/10.1016/j.jff.2017.05.039

Leßig J., Fuchs B. (2010) HOCl-mediated glycerophosphocholine and glycerophosphoethanolamine generation from plasmalogens in phospholipid mixtures. *Lipids* **45**, 37–51. https://doi.org/10.1007/s11745-009-3365-8

Modica-Napolitano J. S., Renshaw P. F. (2004) Ethanolamine and phosphoethanolamine inhibit mitochondrial function in vitro: Implications for mitochondrial dysfunction hypothesis in depression and bipolar disorder. *Biol. Psychiatry*. **55**, 273-277. https://doi.org/10.1016/S0006-3223(03)00784-4

Nelson C., Moffat B., Jacobsen N., Henzel W. J., Stults J. T., King K. L., McMurtrey A., Vandlen R., Spencer S. A. (1996) Glycerophosphorylethanolamine (GPEA) identified as an hepatocyte growth stimulator in liver extracts. *Exp. Cell Res.* **229**, 20–26. https://doi.org/10.1006/excr.1996.0339

Rakhit S., Bagli J. F., Deghenghi R. (1969) Phospholipids. Part I. Synthesis of phosphatidyl ethanolamines. *Can. J. Chem.* **47**, 2906-2910. https://doi.org/10.1139/v69-486

Tallan H. H., Moore S., Stein W. H. (1954) Studies on the free amino acids and related compounds in the tissues of the cat. *J. Biol. Chem.* **211**, 927-939.

Tables:

Table 1: Direct hydrolysis of DPPE under various alkali conditions to obtain GroPEtn

[*1 mg of DPPE dissolved in chloroform: methanol (1:1) is used in each reaction.

Reaction status in each step monitored by TLC and "X"-refers to no reaction].

SN	Reagent*	Temperature	Time (hr)	Status#
1	1 M aq. KOH	RT	12	X
2	1M aq. KOH	60 ºC	1	byproducts
3	NaOMe (1.1 eq)	RT	0.5	byproducts
4	1M aq. LiOH	RT	12	X
5	K_2CO_3 (2 eq)	RT	12	X
6	$LiCO_3$ (2 eq)	RT	12	X

Figure legends:

Figure 1: Semi-synthetic approach for efficient synthesis of GroPEtn from DPPE

Figure 2. Diagrammatic representation of hydrolysis of Tr-GroPEtn to GroPEtn and its mass spectrometric characterization. **A.** Pictorial representation of reaction process and detection of GroPEtn by TLC. **B.** Characterization of GroPEtn by linear trap quadrupole-Orbitrap mass spectrometry [* TLC elution gradient is: EtOAc:MeOH:H_2O:CH_3COOH (2:1:1:0.3)]

Figure 3. ^{1}H and ^{13}C- NMR spectra of GroPEtn

Figure 4. A. Cell viablity assay results of GroPEtn treated with HK-2 cells and **B**.Reporter gene assay results of GroPEtn in Nrf2 activation.

Graphical Abstract:

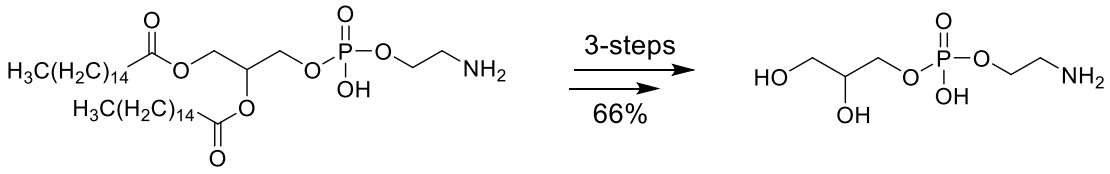


Figure 1:

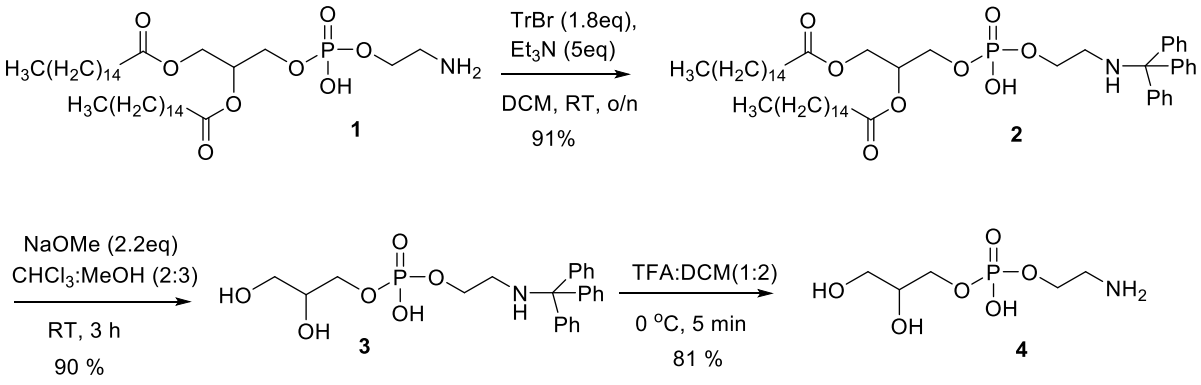


Figure 2.

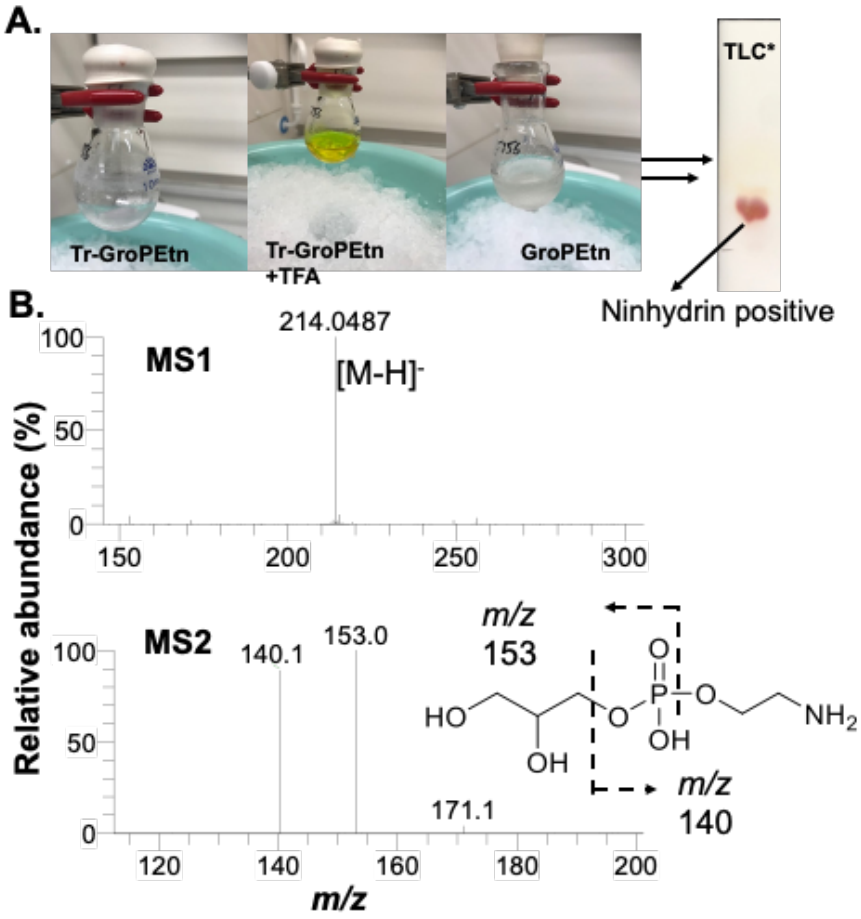
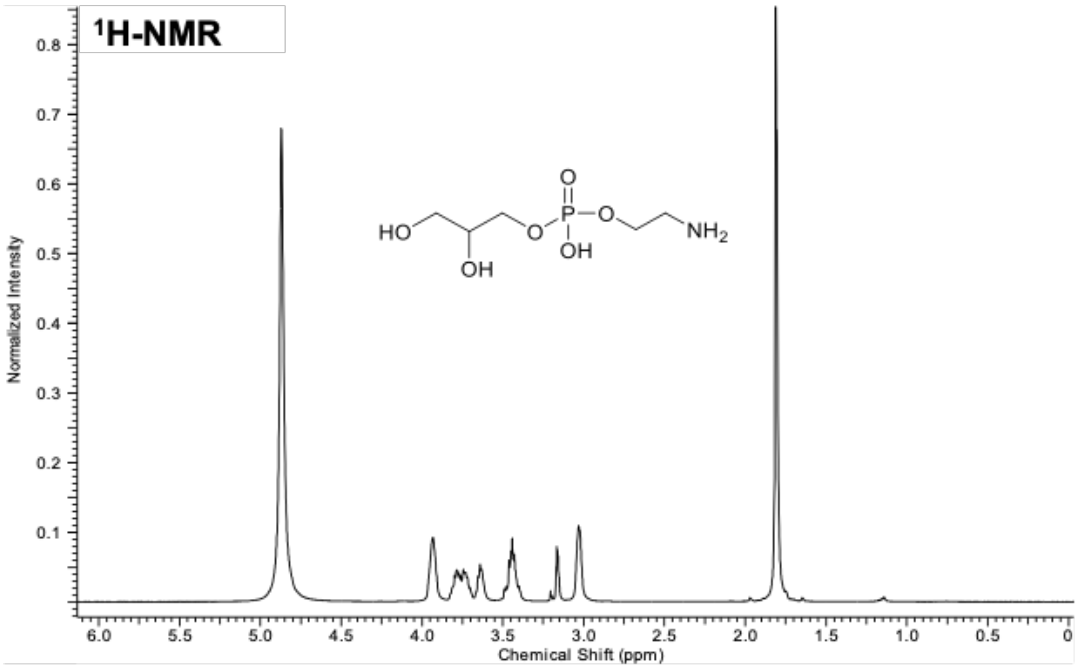


Figure 3.


¹H-NMR
Normalized Intensity
Chemical Shift (ppm)
HO
OH
O
P
O
OH
NH2

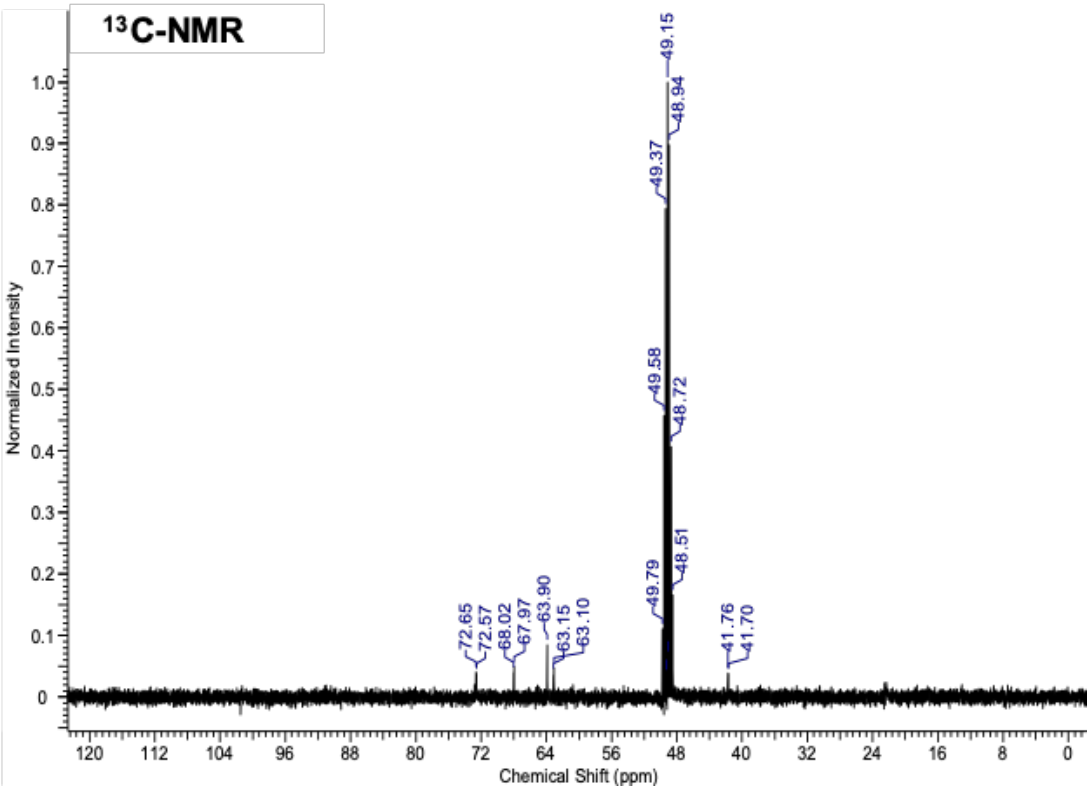
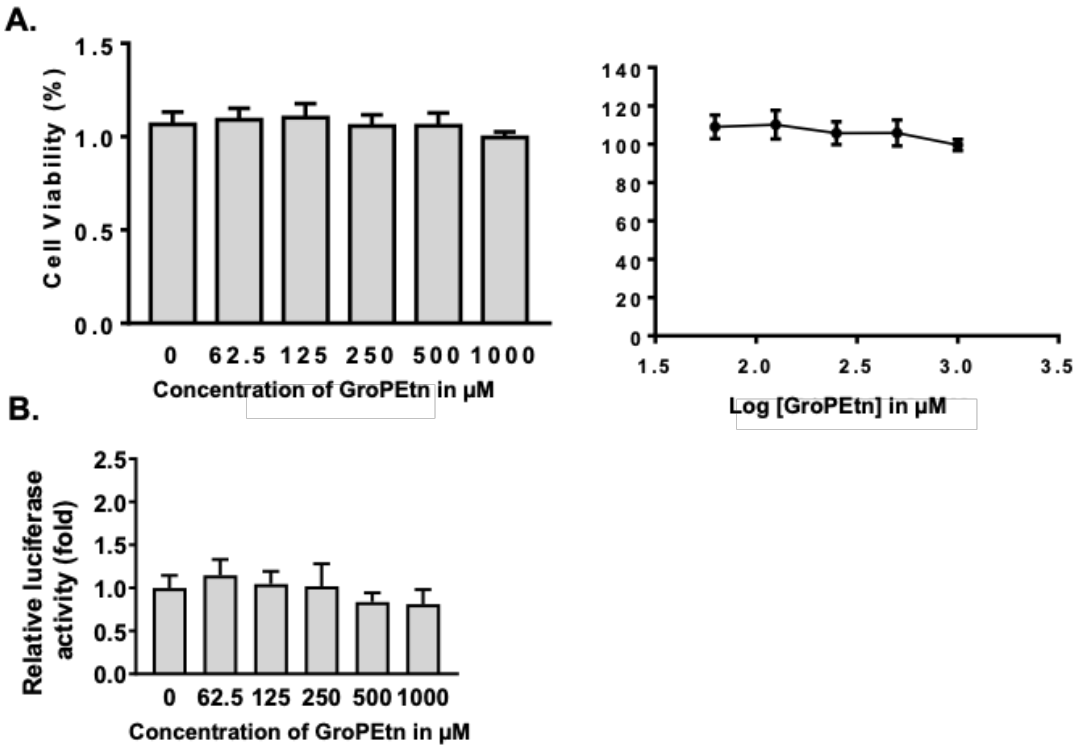

¹³C-NMR
Normalized Intensity
Chemical Shift (ppm)
49.15
48.94
49.37
49.58
48.72
48.51
49.79
72.65
72.57
68.02
67.97
63.90
63.15
63.10
41.76
41.70

Figure 4.


A.
Cell Viability (%)
1.5
1.0
0.5
0.0
0
62.5
125
250
500
1000
Concentration of GroPEtn in µM
140
120
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log [GroPEtn] in µM
B.
Relative luciferase activity (fold)
2.5
2.0
1.5
1.0
0.5
0.0
0
62.5
125
250
500
1000
Concentration of GroPEtn in µM

Supporting Information for

A simple and efficient method for synthesis of Sn-Glycero-phosphoethanolamine

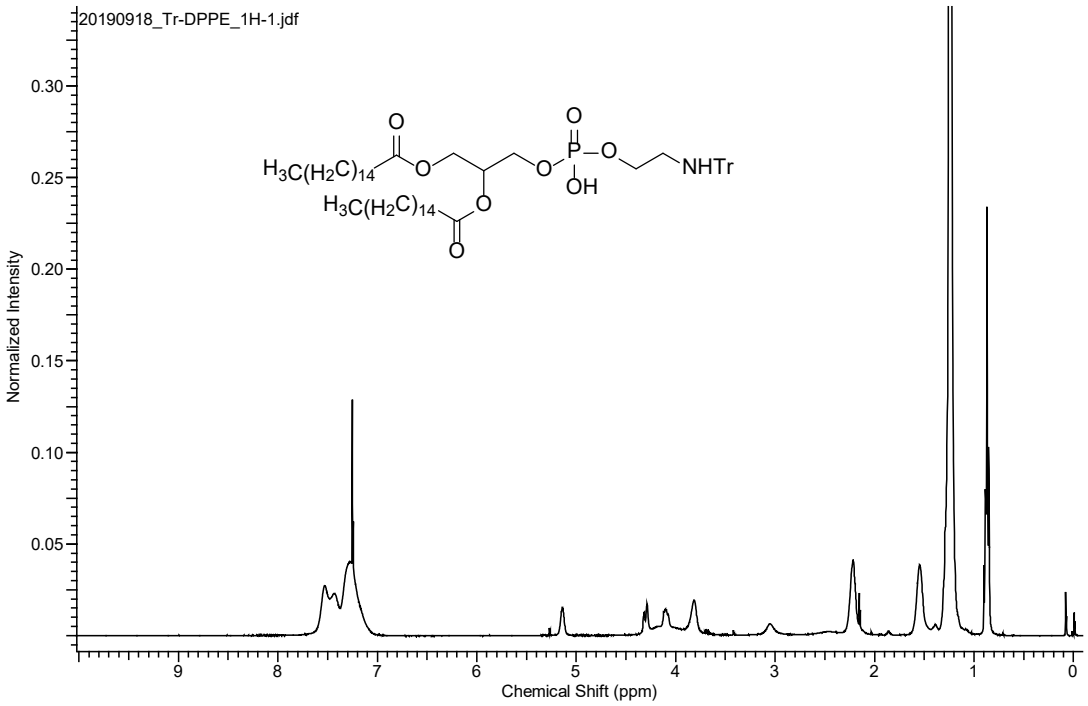
Siddabasave Gowda B. Gowda[1], Hirotoshi Fuda[1], Yusuke Yamamoto[2], Hitoshi Chiba[3], and Shu-Ping Hui[1*]

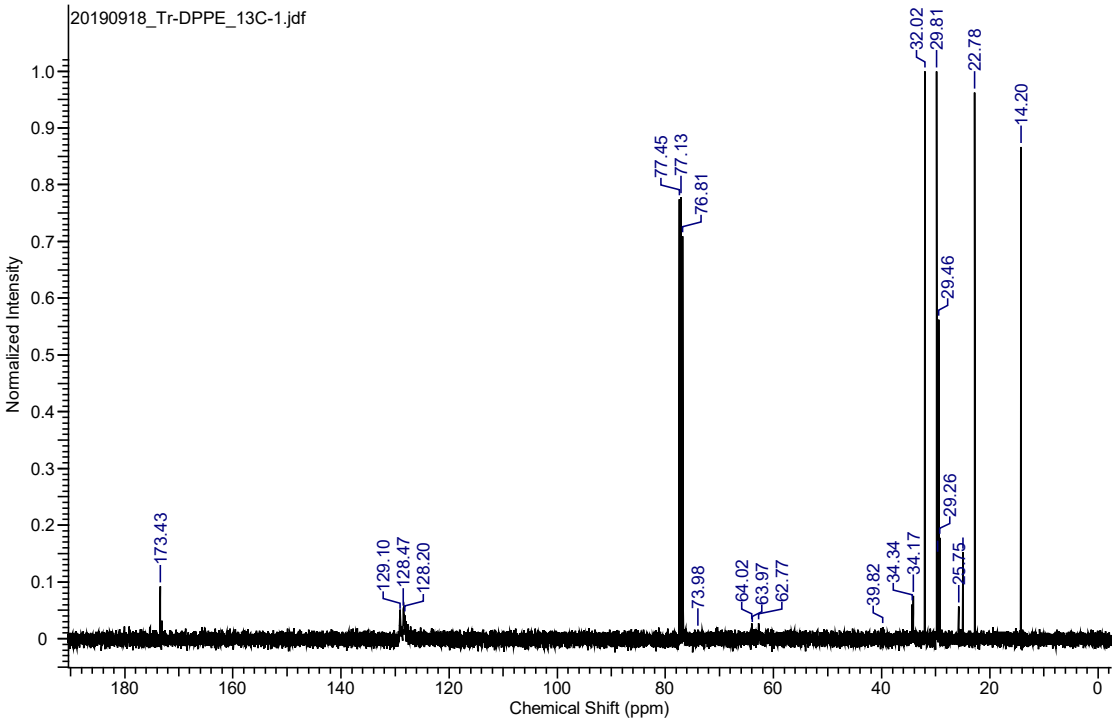
1. Faculty of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan
2. Graduate School of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan
3. Department of Nutrition, Sapporo University of Health Sciences, Nakanuma, Nishi 4-2-1-15, Higashi-ku, Sapporo 007-0894, Japan

Table of contents:

Sl	Title	Page no
1	1H and ^{13}C-NMR of Tr-DPPE (2)	S2
2	^{1}H and ^{13}C-NMR of Tr-GroPEtn (3)	S3

1. 1H and ^{13}C-NMR of Tr-DPPE (2)





2. ^{1}H and ^{13}C-NMR of Tr-GroPEtn (3)