Bioscience, Biotechnology, and Biochemistry, 2021, Vol. 85, No. 6, 1383-1389

doi: 10.1093/bbb/zbab037
Advance access publication date: 11 March 2021
REGULAR PAPER

REGULAR PAPER

Synthesis of a plasmenylethanolamine

Satoshi Maeda,[1] Tomoyo Mohri,[2] Tsubasa Inoue,[1] Yoshio Asano,[1] Yurika Otoki,[1] Masaru Enomoto,[2] Kiyotaka Nakagawa,[1] Shigefumi Kuwahara,[2] and Yusuke Ogura[2,3,*]

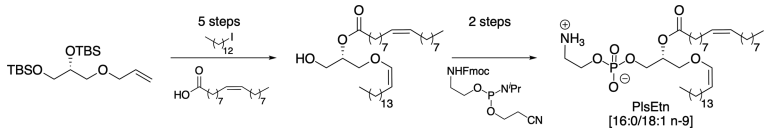
[1]Laboratory of Food and Biodynamic Chemistry, Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai, Japan; [2]Laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai, Japan; and [3]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

*Correspondence: Yusuke Ogura, ayskogura@g.ecc.u-tokyo.ac.jp

ABSTRACT

A concise synthesis of a plasmenylethanolamine (PlsEtn-[16:0/18:1 n-9]), known as antioxidative phospholipids commonly found in cell membranes, has been achieved from an optically active known diol through 8 steps. The key transformations for the synthesis of PlsEtn-[16:0/18:1 n-9] are (1) regio- and *Z*-selective vinyl ether formation via the alkylation of a lithioalkoxy allyl intermediate with an alkyl iodide, and (2) a one-pot phosphite esterification–oxidation sequence to construct the ethanolamine phosphonate moiety in the presence of the vinyl ether functionality. The piperidine salt of synthetic PlsEtn-[16:0/18:1 n-9] was desalinated through reversed-phase high-performance liquid chromatography purification.

Graphical Abstract



Synthesis of a plasmenylethanolamine-[16:0/18:1 n-9].

Keywords: plasmalogen, total synthesis, plasmenylethanolamine, natural product, vinyl ether

Plasmenyl phospholipids (also called plasmalogens) are a subclass of naturally occurring glycerophospholipids, which comprise about 20% of the total phospholipids in human (Horrocks and Sharma 1982; Nagan and Zeoller 2001; Engelmann 2004). They structurally feature an *O*-(*Z*)-alkenyl ether moiety (usually C16:0, C18:0, or C18:1) at the *sn*-1 position and a (poly)unsaturated fatty acyl chain at the *sn*-2 position, and are mainly classified into 2 groups, plasmenylethanolamine (PlsEtn) and plasmenylcholine (PlsCho), based on the structure of the head group at the *sn*-3 position (Figure 1) (Brites, Waterham and Wanders 2004; Braverman and Moser 2012).

These structurally intriguing phospholipids are predominantly found in cell membrane (Klenk and Debuch 1963; Horrocks and Sharma 1982) of mammalian tissues such as heart (Gross 1984; Gross 1985), brain (Snipes and Suter 1997),

Received: 17 February 2021; **Accepted:** 5 March 2021

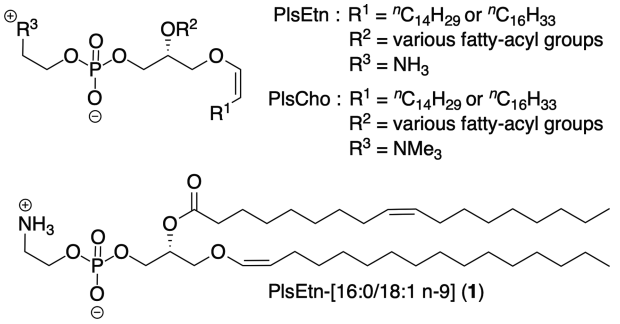
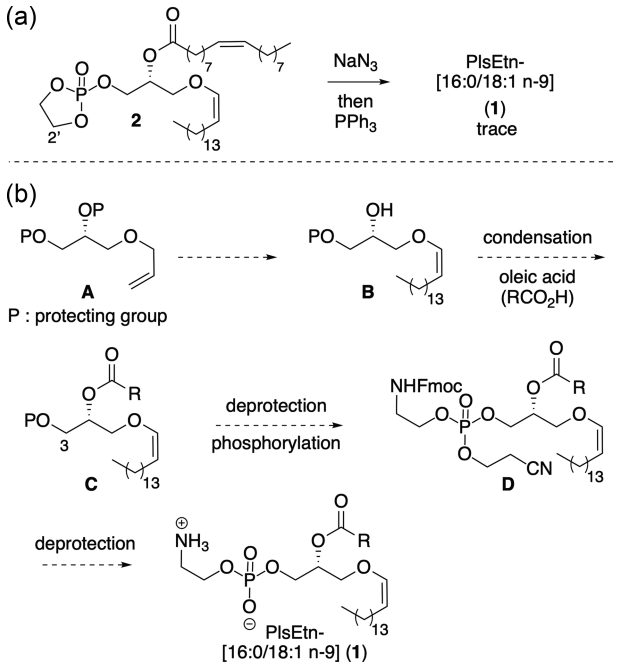


Figure 1. Structures of typical plasmenyl phospholipids (plasmalogens) and the synthetic target in this work, PlsEtn-[16:0/18:1 n-9] (**1**).

and myelin (Sugiura and Waku 1987). Additionally, plasmalogens are also found in kidney (Braverman and Moser 2012), muscle (Horrocks and Sharma 1982; Braverman and Moser 2012), as well as blood (Braverman and Moser 2012). Moreover, these phospholipids are also abundant in marine invertebrates as well as anaerobic bacteria (Goldfine 2010; Yamashita *et al.* 2016). As the plasmalogens are thought to play roles in many important biological processes such as membrane fusion (Hermetter *et al.* 1989; Glaser and Gross 1994; Thompson *et al.* 1996) and signal transduction (Fonteb and Chilton 1992; Snyder, Lee and Blank 1997) including supply of polyunsaturated fatty acids (Ford and Gross 1989) leading to production of some bioactive compounds (Farooqui, Yang and Horrocks 1995; Portilla and Creer 1995; McHowat, Liu and Creer 1998; Murphy 2001), a number of biologists related to the research on have been intrigued in clarifying the details of their biochemical and biophysical properties. In addition, these phospholipids are known to act as antioxidants (Zoeller, Morand and Raetz 1988; Reiss, Beyer and Engelmann 1997). Furthermore, it is also revealed that the level of PlsEtn decreases in the brain with neurodegenerative disorders such as Alzheimer's disease (Farooqui and Horrocks 1998; Ginsberg, Xuereb and Gershfeld 1998; Goodenowe *et al.* 2007; Wood *et al.* 2010; Oma *et al.* 2012; Yamashita *et al.* 2015), which has continuously attracted much interest on PlsEtn not only from biologists but also from biochemists and medicinal chemists.

The structurally and biologically intriguing profiles of plasmalogens, especially PlsEtn, as described above have also stimulated synthetic chemists as well as enzyme and biosynthetic chemists. The enzymatic syntheses of PlsEtn have frequently been studied from 1970s (Blank, Wykle and Snyder 1971; Snyder, Blank and Wykle 1971; Paltauf and Holasek 1973), which culminated in the development of some methods to produce PlsEtn as a mixture of some plasmalogen molecular species. Semisynthetic approaches were also developed (Hermetter and Paltauf 1982), which allowed to provide some PlsEtn molecular species in pure state. However, the variety, purity, and/or quantity of the synthetic PlsEtn molecular species produced by these methods have still left room for improvement. Moreover, any solid method for the isolation, separation, and purification of each discrete PlsEtn molecular species from natural sources (eg sea squirt, sea urchin, and shellfish, in which PlsEtn are known to be abundant) has not been well established yet. Thus, the focus of the method to supply various species of PlsEtn in pure state has shifted to chemical synthetic approaches. However, despite the huge attention to this type of bioactive glycerophospholipids from a wide range of scientific fields, only 2 synthetic routes for PlsEtn have been reported (Pfaendler and Weimar 1996; Khan, Wood and Goodenowe 2015), while 5 total syntheses of PlsCho have been disclosed (Rui and Thompson 1996; Qin,



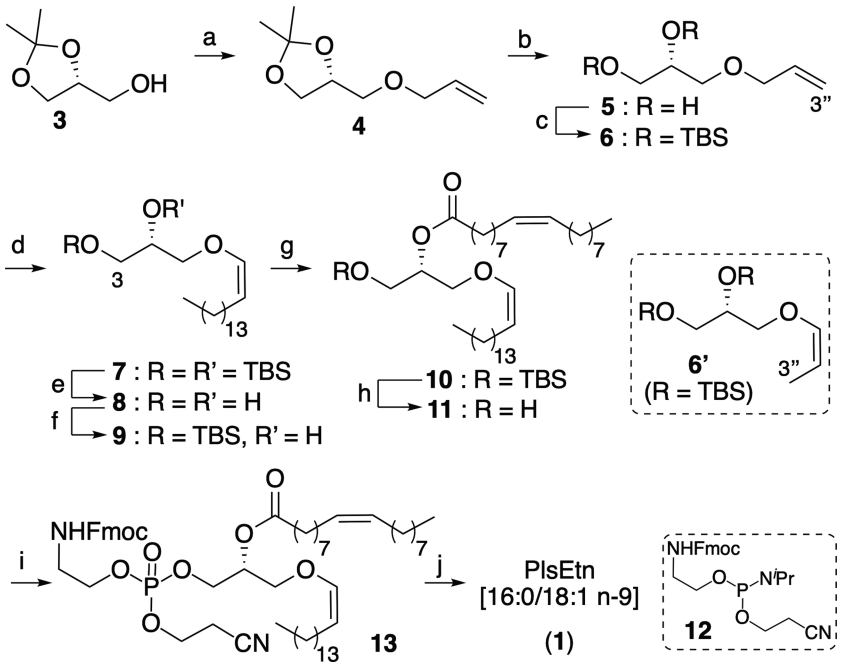
Scheme 1. Our initial attempt of the synthesis of **1** (a) and alternative synthetic plan for PlsEtn-[16:0/18:1 n-9] (b).

Byun and Bittman 1999; Shin *et al.* 2001; Shin, Gerasimov and Thompson 2002; Shin and Thompson 2003; Van den Bossche, Shin and Thompson 2007). In this article, we describe a new synthesis of a plasmenylethanolamine via a one-pot phosphite esterification–oxidation sequence to construct the ethanolamine phosphonate moiety, which would also be applicable to the synthesis of many other PlsEtn molecular species. For the establishment of the synthetic route for various kinds of PlsEtn, we chose PlsEtn-[16:0/18:1 n-9] (**1**) as a representative synthetic target (Figure 1).

Results and discussion

Initially, the synthesis of **1** was performed in accordance with a previous report (Rui and Thompson 1996) on the synthesis of PlsCho (Scheme 1a). Unfortunately, however, the transformation of cyclic phosphonate **2**, obtained in line with the report, to PlsEtn-[16:0/18:1 n-9] (**1**) via nucleophilic ring-opening reaction at the C2' position utilizing sodium azide followed by reduction of the corresponding azide group with PPh_3 yielded only a trace amount of desired **1**. Faced with this undesired outcome, we made an alternative synthetic plan for **1** as depicted in Scheme 1b. First, the key intermediate **B** bearing (*Z*)-vinyl ether group would be preparable in a regio- and stereoselective manner by alkylation of a lithioalkoxy allyl intermediate derived from **A** with 1-iodotridecane (Shin and Thompson 2003). Condensation of the secondary alcohol **B** with oleic acid would afford fatty acid ester **C**. Deprotection of **C** should give a primary alcohol intermediate, the phosphorylation of which would deliver phosphate **D** by using phosphoramidite chemistry (Swarts and Guo 2010). Finally, global deprotection of **D** would complete the synthesis of PlsEtn-[16:0/18:1 n-9] (**1**).

The synthesis of PlsEtn-[16:0/18:1 n-9] (**1**) following the new synthetic plan commenced with the preparation of known optically active diol **5** (Mikkilineni, Kumar and Abushanab 1988) (Scheme 2). The sodium alkoxide of commercially available (*R*)-glycerol acetonide **3** was prepared by its treatment with sodium



Scheme 2. Synthesis of PlsEtn-[16:0/18:1 n-9] (**1**). Reagents and conditions: (a) allyl bromide, NaH, DMF, rt, 2 h, 82%; (b) Amberlite IR-120 hydrogen form, EtOH, 40 °C, overnight, 93%; (c) TBSCl, imidazole, DMF, 0 °C to rt, overnight, 68% (d) *s*-BuLi, THF −78 °C, 0.5 h, then 1-iodotridecane, −78 °C to rt, overnight, 39%; (e) TBAF, THF, rt, 12 h, 94%; (f) TBSCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, overnight, 86%; (g) oleic acid, EDC·Cl, DMAP, CH_2Cl_2, rt, overnight, 97%; (h) TBAF/AcOH (pH = 4.4-4.7), THF, rt, overnight, 61%; (i) **12**, 1*H*-tetrazole, MeCN, then TBHP, rt, 0.5 h, 75%; (j) piperidine, DMF, 0 °C to rt, overnight, 53%.

hydride and alkylated by allyl bromide to afford allyl ether **4**. Exposure of **4** to acidic conditions in ethanol effected deprotection of the acetonide group to provide diol **5** in excellent yield, which was then protected as bis-TBS ether **6**. The *Z*-configurated vinyl ether moiety characteristic of plasmalogen was successfully constructed in a stereoselective manner by applying the Thompson's procedure (Shin, Gerasimov and Thompson 2002). Exposure of **6** to *s*-butyllithium to generate a lithioalkoxy allyl intermediate was followed by treatment with 1-iodotridecane to effect alkylation at C3′′ to give **7**, albeit in a moderate yield of 39% mainly due to the formation of an undesired product **6′** protonated at C3" (ca. 15%). Another procedure using BaI_2 as a chelating additive (Van den Bossche, Shin and Thompson 2007) resulted, in our hands, in a much lower yield of **7** (6.0%). The desired **7** could be purified by standard silica gel chromatography and stored in a freezer at low temperature of −20 °C for months without any degradation. The (*Z*)-vinyl ether **7** was converted to secondary alcohol **9** via a two-step sequence composed of deprotection of the two TBS groups with TBAF and subsequent regioselective TBS-protection of primary hydroxy group at C3 of the resulting diol **8**. The secondary hydroxy group of **9** was then subjected to acylation with oleic acid under standard conditions utilizing EDC·HCl in the presence of DMAP to provide oleate ester **10** in an excellent yield of 97%. Treatment of **10** with TBAF in THF unexpectedly brought about not only deprotection of TBS group to afford desired product **11** but also migration of the *sn*-2 acyl group of **11** to the *sn*-3 position (64% yield), causing severe decrease in the yield of **11** (16% yield). Utilizing TASF as an anhydrous source of fluoride anion resulted in a much lower yield of **11** (6.4%). On the other hand, exposure of **10** to a solution of TBAF weakly acidified with AcOH (pH = 4.4) suppressed the problematic migration and thereby significantly improved the yield of **11** to 61%. The phosphorylation of the primary alcohol **11** was successfully performed by condensation of **11** with phosphoramidite **12** to generate a phosphite intermediate followed by its *in situ* oxidation with *t*-BuOOH to furnish protected ethanolamine phosphonate **13** in a satisfactory yield of 75% (Swarts and Guo 2010) without causing any oxidative degradation of the potentially oxidant-sensitive vinyl ether moiety. Finally, deprotection of the Fmoc and cyanoethyl groups of **13** with an excess amount of piperidine in DMF furnished PlsEtn-[16:0/18:1 n-9] (**1**) as a piperidine salt, which was desalinated through purification by reversed-phase high-performance liquid chromatography (HPLC) (COSMOSIL $5C_{18}$-MS-II; MeOH) to complete the synthesis of PlsEtn-[16:0/18:1 n-9] (**1**). The ^1H and ^{13}C NMR data as well as the optical rotation of synthetic **1** were in good accordance with those reported (Morandat et al. 2003). The MS/MS analysis of synthetic **1** also showed some characteristic fragment ions of PlsEtn (see Supporting Information) (Otoki *et al.* 2015).

Conclusion

In conclusion, a concise synthesis of PlsEtn-[16:0/18:1 n-9] (**1**) was achieved in 8 steps from known optically active diol **5**. The (*Z*)-configurated vinyl ether moiety of **1** was constructed via regio- and *Z*-selective alkylation of a lithioalkoxy allyl intermediate with 1-iodotridecane. The installation of the protected ethanolamine phosphonate moiety in the presence of a vinyl ether group was successfully affected by a one-pot phosphite formation–oxidation sequence. The synthetic piperidine salt of **1** was desalinated through purification by reversed-phase HPLC to afford salt-free PlsEtn-[16:0/18:1 n-9] (**1**). The synthetic PlsEtn-[16:0/18:1 n-9] (**1**) will be useful as a standard for advanced researches on the clarification of new physiological functions of plasmalogens. Synthetic efforts toward other members of this class of natural products are also in progress and will be reported in due course.

Experimental

General procedure

IR spectra were recorded by a Jasco FT/IR-4100 spectrometer using an ATR (ZnSe) attachment. NMR spectra were recorded with TMS as an internal standard in $CDCl_3$ by a Varian 400-MRTT spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C), a Varian 600TT spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C) or a JMN-ECA800 (320 MHz for ^{31}P) unless otherwise stated. Chemical shifts (δ) of ^{13}C NMR and ^{31} P NMR spectra were reported relative to $CDCl_3$ (δ 77.00) and H_3PO_4 (δ 0.00), respectively. Optical rotation values were measured with a Jasco P-2200 polarimeter. Mass spectra were obtained with Jeol JMS-700 spectrometer operated in the EI or FAB mode. Kanto Kagaku silica gel 60N (100-210 μm) was used for column chromatography unless otherwise stated. Analytical thin-layer chromatography was performed using Merck silica gel 60 F_{254} plates (0.25 mm thick). Solvents for reactions were distilled prior to use: THF from Na and benzophenone; CH_2Cl_2 and DMF from CaH_2. All air- or moisture-sensitive reactions were conducted under a nitrogen atmosphere unless otherwise stated.

(*R*)-(−)-2,2-Dimethyl-1,3-dioxolane-4-[(allyloxy)methyl] (4)

NaH (60% in mineral oil suspension, 0.623 g, 15.6 mmol) was washed 3 times with hexane under a nitrogen atmosphere and suspended in dry DMF (75.7 mL). To the suspension, (*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol (**3**) (1.00 g, 7.60 mmol) was slowly added while stirring at ambient temperature. After 5 min, allyl bromide (1.28 mL, 15.2 mmol) was slowly added to the pale yellow suspension below 30 °C (water bath). After stirring for 2 h, the reaction mixture was quenched with water while cooling in an ice bath and extracted 3 times with EtOAc. The extract was successively washed with water and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by SiO_2 column chromatography (hexane/EtOAc = 8:1) to give **4** (1.07 g, 82%) as a pale yellow oil. $[\alpha]^{24}_D$ −18.1 (*c* 0.375, $CHCl_3$); IR: ν_{max} 3019 (w), 2925 (s), 2854 (m), 1261 (m); ^1H NMR (400 MHz, $CDCl_3$): δ 1.37 (3H, s), 1.43 (3H, s), 3.45 (1H, dd, J = 5.5, 9.8 Hz), 3.53 (1H, dd, J = 5.8, 9.8 Hz), 3.74 (1H, dd, J = 6.4, 8.2 Hz), 4.03-4.09 (3H, m), 4.29 (1H, quin, J = 6.0 Hz), 5.23 (1H, d, J = 10.5 Hz), 5.25 (1H, d, J = 17.2 Hz), 5.91 (1H, ddt, J = 10.5, 17.2, 5.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.4, 26.8, 66.8, 71.1, 72.5, 74.7, 109.4, 117.4, 134.5; HRMS (ESI): *m/z* calcd for $C_9H_{16}NaO_3$, 195.0997; found, 195.0997 ($[M + Na]^+$).

(*S*)-1-(Allyloxy) propane-2,3-diol (5)

To a stirred solution of **4** (24.6 g, 143 mmol) in EtOH (99.5%, 1.40 L) was added Amberlite IR-120 hydrogen form (84.4 g) at ambient temperature, and the mixture was warmed to 40 °C. After stirring overnight at the same temperature, the reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in $CHCl_3$ and the solution was dried ($MgSO_4$), filtered and concentrated in vacuo to give **5** (17.2 g, 93%) as a pale yellow oil. $[\alpha]^{24}_D$ + 3.16 (*c* 0.285, $CHCl_3$); IR: ν_{max} 3380 (br), 3013 (w), 1645 (w), 1422 (m), 1041 (s); ^1H NMR (400 MHz, $CDCl_3$): δ 2.14 (1H, s), 2.61 (1H, s) 3.52 (1H, dd, J = 6.2, 9.7 Hz), 3.56 (1H, dd, J = 3.9, 9.7 Hz), 3.62-3.77 (2H, m), 3.86-3.92 (1H, m), 4.03 (2H, d, J = 5.7 Hz), 5.20-5.23 (1H, m), 5.21 (1H, d, J = 10.4 Hz), 5.28 (1H, d, J = 17.2 Hz), 5.90 (1H, ddt, J = 10.4, 17.2, 5.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 64.1, 70.6, 71.8, 72.5, 117.6, 134.2; HRMS (FAB): *m/z* calcd for $C_6H_{12}NaO_3$, 155.0679; found, 155.0685 ($[M + Na]^+$).

(*R*)-5-((Allyloxy)methyl)-octamethyl-4,7-dioxa-3, 8-disiladecane (6)

To a stirred solution of **5** (3.90 g, 29.5 mmol) in dry DMF (295 mL) was added imidazole (2.88 g, 42.3 mmol) and TBSCl (12.9 g, 85.3 mmol) at 0 °C and the reaction mixture was warmed to ambient temperature. After stirring overnight, the mixture was quenched with sat aq. $NaHCO_3$ while cooling in an ice bath and extracted 3 times with Et_2O. The extract was successively washed with water and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by SiO_2 column chromatography (hexane/EtOAc = 50:1) to give **6** (7.23 g, 68%) as a pale yellow oil. $[\alpha]^{27}_D$ −0.149 (*c* 1.00, $CHCl_3$); IR: ν_{max} 3014 (w), 1471 (m), 1255 (m), 1108 (m); ^1H NMR (400 MHz, $CDCl_3$): δ .05 (6H, s), .08 (6H, s), 0.89 (18H, s), 3.37 (1H, dd, J = 5.7, 9.9 Hz), 3.50 (1H, dd, J = 4.6, 9.9 Hz), 3.54 (1H, dd, J = 5.6, 10.2 Hz), 3.59 (1H, dd, J = 5.9, 10.2 Hz), 3.79-3.87 (1H, m), 3.99 (2H, dt, J = 1.5, 5.5 Hz), 5.15 (1H, ddd, J = 1.5, 3.3, 10.4 Hz), 5.26 (1H, ddd, J = 1.7, 3.4, 17.3 Hz), 5.90 (1H, ddt, J = 10.4, 17.3, 5.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.4 (2C), −4.7, −4.6, 18.2 (3C), 18.3 (3C), 25.6, 25.9, 65.1, 72.2, 72.3, 72.7, 116.5, 135.0; HRMS (FAB): *m/z* calcd for $C_{18}H_{41}O_3Si_2$, 361.2589; found, 361.2596 ($[M + H]^+$).

(*R*,*Z*)-5-((Hexadec-1-en-1-yloxy)methyl)-2,2,3,3,8,8,9, 9-octamethyl-4,7-dioxa-3,8-disiladecane (7)

To a stirred solution of **6** (1.00 g, 2.78 mmol) in dry THF (27.7 mL), *s*-BuLi (1.0 M in cyclohexane/hexane, 4.05 mL, 4.05 mmol) was added dropwise at −78 °C under a nitrogen atmosphere. After stirring for 30 min at the same temperature, a solution of 1-iodotridecane (2.25 g, 7.26 mmol) in dry THF (11.0 mL) was added and the reaction mixture was warmed to ambient temperature and stirred overnight. The reaction mixture was quenched with water while cooling in an ice bath and extracted 3 times with Et_2O. The extract was successively washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by SiO_2 column chromatography (hexane/CH_2Cl_2 = 20:1) to give **7** (0.59 g, 39%) as a pale yellow oil. $[\alpha]^{25}_D$ −6.58 (*c* 1.00, $CHCl_3$); IR: ν_{max} 3034 (w), 1659 (m), 1460 (m), 1253 (m), 1105 (s); ^1H NMR (400 MHz, $CDCl_3$): δ .05 (6H, s), .08 (6H, s), 0.86-0.89 (21H, m), 1.26 (24H, m), 2.01-2.09 (2H, m), 3.53-3.55 (2H, m), 3.64 (1H, dd, J = 6.1, 10.4 Hz), 3.77-3.85 (2H, m), 4.28 (1H, q, J = 6.9 Hz), 5.92 (1H, d, J = 6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.5, −5.4, −4.8, −4.7, 14.1, 18.1 (3C), 18.3 (3C), 22.7, 24.1, 25.8, 25.9, 29.4 (2C), 29.6, 29.67, 29.68, 29.71 (4C), 29.9, 31.9, 64.7, 72.5, 74.2, 106.3, 145.5; HRMS (FAB): *m/z* calcd for $C_{31}H_{67}O_3Si_2$, 543.4623; found, 543.4627 ($[M + H]^+$).

(*S*,*Z*)-3-(Hexadec-1-en-1-yloxy) propane-1,2-diol (8)

To the bis-TBS ether **7** (3.46g, 6.38 mmol) was added TBAF (1.0 M in THF, 25.5 mL, 25.5 mmol) at ambient temperature and the reaction mixture was stirred for 12 h. The reaction mixture was quenched with sat aq. NH_4Cl and extracted 3 times with Et_2O. The extract was successively washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by SiO_2 column chromatography (hexane/EtOAc = 1:1) to give **8** (1.88 g, 94%) as a white solid. $[\alpha]^{25}_D$ −2.38 (*c* 1.00, $CHCl_3$); IR: ν_{max} 3280 (br), 3022 (w), 2848 (s), 1664 (m), 1467 (m), 1364 (s), 1118 (s); ^1H NMR (400 MHz, $CDCl_3$): δ .88 (3H, t, J = 6.5 Hz), 1.26

(24H, s), 1.97 (1H, m), 2.05 (2H, q, J = 7.1 Hz), 2.42-2.48 (1H, m), 3.62-3.70 (1H, m), 3.72-3.84 (3H, m), 3.89-3.98 (1H, m), 4.41 (1H, q, J = 6.8 Hz), 5.94 (1H, dd, J = 1.3, 6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 23.9, 29.3, 29.4, 29.6, 29.65, 29.67, 29.70 (4C), 29.72, 31.9, 63.6, 70.8, 73.0, 108.2, 144.5; HRMS (EI): m/z calcd for $C_{19}H_{38}O_3$, 314.2821; found, 314.2821 ([M]$^+$).

(*R*,*Z*)-1-((tert-Butyldimethylsilyl)oxy)-3-(hexadec-1-en-1-yloxy) propan-2-ol (9)

To a stirred solution of DMAP (0.140 g, 1.14 mmol), TBSCl (0.345 g, 2.29 mmol) and Et_3N (318 µL, 2.29 mmol) in CH_2Cl_2 (5.50 mL) was added a solution of **8** (0.300 g, 0.954 mmol) in CH_2Cl_2 (1.66 mL) at 0 °C. After 5 h, the reaction mixture was quenched with water while cooling in an ice bath and extracted 3 times with CH_2Cl_2. The extract was successively washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by SiO_2 column chromatography (hexane/EtOAc = 10:1) to give **9** (0.323 g, 86%) as a pale yellow oil. $[\alpha]^{25}_D$ −4.74 (c 1.00, $CHCl_3$); IR: ν_{max} 3454 (br), 3030 (w), 1665 (m), 1465 (m), 1255 (m), 1105 (m); ^{1}H NMR (400 MHz, $CDCl_3$): δ .08 (6H, s), 0.90 (12H, m), 1.26 (24H, m), 2.05 (2H, dq, J = 1.1, 7.2 Hz), 2.41 (1H, d, J = 5.6 Hz), 3.66 (1H, dd, J = 5.4, 10.1 Hz), 3.69 (1H, dd, J = 4.7, 10.1 Hz), 3.73-3.78 (2H, m), 3.80-3.87 (1H, m), 4.36 (1H, q, J = 6.9 Hz), 5.94 (1H, dt, J = 1.5, 6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.5 (2C), 14.1, 18.3 (3C), 22.7, 23.9, 25.8, 29.3, 29.4, 29.5, 29.65, 29.66, 29.69 (4C), 29.8, 31.9, 63.5, 70.5, 72.3, 107.7, 144.8; HRMS (EI): m/z calcd for $C_{25}H_{52}O_3Si$, 428.3686; found, 428.3689 ([M]$^+$).

(*R*)-1-((tert-Butyldimethylsilyl)oxy)-3-(((*Z*)-hexadec-1-en-1-yl)oxy)propan-2-yl oleate (10)

To a stirred solution of oleic acid (1.77 M in EtOH, 1.27 mL, 2.25 mmol) and DMAP (0.276 g, 2.26 mmol) in CH_2Cl_2 (6.0 mL), EDC·HCl (0.651 g, 3.40 mmol) was added at ambient temperature. After 30 min, **9** (0.486 g, 1.13 mmol) in CH_2Cl_2 (5.0 mL) was added and resulting mixture was stirring overnight. The mixture was quenched with water while cooling in an ice bath and extracted 3 times with CH_2Cl_2. The extract was successively washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by SiO_2 column chromatography (hexane/EtOAc = 20:1) to give **10** (0.765 g, 97%) as a pale yellow oil. $[\alpha]^{25}_D$ + 3.64 (c 1.00, $CHCl_3$); IR: ν_{max} 3004 (w), 2853 (s), 1741 (s), 1665 (m), 1464 (m), 1253 (m), 1108 (s); ^{1}H NMR (400 MHz, $CDCl_3$): δ .06 (6H, s), 0.86-0.90 (15H, m), 1.26-1.30 (44H, m), 1.58-1.68 (2H, m), 1.97-2.08 (6H, m), 2.31 (2H, t, J = 7.5 Hz), 3.72 (1H, dd, J = 5.1, 10.8 Hz), 3.75 (1H, dd, J = 5.2, 10.8 Hz), 3.84 (1H, dd, J = 5.6, 11.3 Hz), 3.89 (1H, dd, J = 4.6, 11.3 Hz), 4.34 (1H, q, J = 6.9 Hz), 5.00 (1H, quin, J = 5.1 Hz), 5.29-5.39 (2H, m), 5.90 (1H, dt, J = 1.4, 6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.5 (2C), 14.1 (2C), 18.2 (3C), 22.7 (2C), 23.9, 24.9, 25.8, 27.2 (2C), 29.1 (2C), 29.2, 29.3 (2C), 29.4, 29.5, 29.6, 29.67, 29.70, 29.72 (6C), 29.8 (2C), 31.91, 31.93, 34.4, 61.3, 70.0, 72.8, 107.6, 129.7, 130.0, 144.9, 173.2; HRMS (EI): m/z calcd for $C_{43}H_{84}O_4Si$, 692.6139; found, 692.6135 ([M]$^+$).

(*S*)-1-(((*Z*)-Hexadec-1-en-1-yl)oxy)-3-hydroxypropan-2-yl oleate (11)

To a stirred solution of TBAF (1.0 M in THF, 0.2 mL, 0.2 mmol), was added AcOH (58.0 µL, 0.966 µmol) to adjust the pH of the solution to 4.4-4.7. The TBAF solution prepared above (0.8 M, 0.14 mL, 0.11 mmol) was added to a solution of **10** (31.6 mg, 0.0456 mmol) in dry THF (0.456 mL) at ambient temperature. After stirring overnight, the reaction mixture was quenched with sat aq. $NaHCO_3$ and extracted 3 times with Et_2O. The extract was successively washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by SiO_2 column chromatography (hexane/EtOAc = 6:1) to give **11** (16.0 mg, 61%) and acyl-migrated product **S1** (2.1 mg, 8%) as a pale yellow oil. $[\alpha]^{25}_D$ −2.66 (c 1.00, $CHCl_3$); IR: ν_{max} 3471 (br), 3005 (w), 2854 (s), 1740 (m), 1668 (m), 1460 (m), 1111 (m); ^{1}H NMR (400 MHz, $CDCl_3$): δ .88 (6H, t, J = 6.8 Hz), 1.26-1.30 (44H, m), 1.58-1.69 (2H, m), 1.89 (1H, t, J = 6.2 Hz), 1.98-2.08 (6H, m), 2.35 (2H, t, J = 7.6 Hz), 3.75-3.86 (2H, m), 3.89 (2H, d, J = 5.2 Hz), 4.39 (1H, q, J = 6.6 Hz), 5.04 (1H, quin, J = 5.2 Hz), 5.28-5.40 (2H, m), 5.91 (1H, d, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (2C), 22.7 (2C), 23.9, 24.9, 27.2 (2C), 29.07, 29.10, 29.2, 29.3 (3C), 29.4, 29.5, 29.6, 29.66, 29.68 (2C), 29.69 (2C), 29.71 (3C), 29.8, 31.90, 31.92, 34.3, 61.9, 70.1, 73.1, 108.2, 129.7, 130.0, 144.5, 173.6; HRMS (FAB): m/z calcd for $C_{37}H_{70}NaO_4$, 601.5166; found, 601.5170 ([M + Na]$^+$).

S1: $[\alpha]^{25}_D$ −2.98 (c 1.04, $CHCl_3$); IR: ν_{max} 3460 (br), 2922 (w), 2852 (m), 1742 (s), 1665 (s), 1465 (s), 1113 (s); ^{1}H NMR (400 MHz, $CDCl_3$): δ .88 (6H, t, J = 6.9 Hz), 1.25-1.30 (44H, m), 1.63 (2H, quint, J = 7.0 Hz), 1.99-2.08 (6H, m), 2.35 (2H, t, J = 7.6 Hz), 2.39 (1H, d, J = 4.9 Hz), 3.74 (1H, dd, J = 6.0, 10.5 Hz), 3.79 (1H, dd, J = 4.6 10.5 Hz), 4.00-4.10 (1H, m), 4.11-4.23 (2H, m), 4.41 (1H, q, J = 7.0 Hz), 5.30-5.39 (2H, m), 5.94 (1H, d, J = 6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (2C), 22.7 (2C), 23.9, 24.9, 27.2 (2C), 29.1 (2C), 29.2, 29.3 (3C), 29.4, 29.5, 29.6, 29.67 (2C), 29.70 (3C), 29.71 (3C), 29.8, 31.91, 31.93, 34.1, 65.1, 70.0, 72.5, 108.5, 129.7, 130.0, 144.4, 174.0; HRMS (FAB): m/z calcd for $C_{37}H_{70}NaO_4$, 601.5166; found, 601.5173 ([M + Na]$^+$).

(2*R*)-1-(((2-((((9H-fluoren-9-yl)methoxy)carbonyl) amino)ethoxy) (2-cyanoethoxy)phosphoryl)oxy)-3-(((*Z*)-hexadec-1-en-1-yl)oxy)propan-2-yl oleate (13)

To a stirred solution of **11** (61 mg, 0.11 mmol) in dry MeCN (5.5 mL), 1*H*-tetrazole (31 mg, 0.44 mmol) was added at 0 °C. After 45 min, a solution of **12** (305 mg, 0.63 mmol) in dry MeCN (6.3 mL) was added at the same temperature and the resulting mixture was warmed to ambient temperature. After checking the consumption of **11** (ca. 1 h), TBHP (5.5 M in decane, 0.29 mL, 1.6 mmol) was added to the reaction mixture. After stirring for 30 min, the mixture was quenched with sat aq. $NaHCO_3$ while cooling in an ice bath and extracted 3 times with EtOAc. The extract was successively washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by SiO_2 column chromatography (hexane/EtOAc = 1:4) to give **13** (77.7 mg, 75%) as a pale yellow oil. $[\alpha]^{24}_D$ −9.33 (c 0.15, $CHCl_3$); IR: ν_{max} 3013 (w), 2853 (m), 1725 (m), 1523 (m), 1451 (m), 1247 (m); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.88 (6H, t, J = 7.1 Hz), 1.25-1.28 (44H, m), 1.57-1.66 (2H, m), 1.97-2.05 (6H, m), 2.32-2.35 (2H, m), 2.72-2.75 (2H, m), 3.50-3.53 (2H, m), 3.81-3.86 (2H, m), 4.16-4.31 (7H, m), 4.38-4.41 (3H, m), 5.19 (1H, quin, J = 5.1 Hz), 5.30-5.37 (2H, m), 5.40-5.50 (1H, m), 5.87 (1H, d, J = 4.1 Hz), 7.32 (2H, t, J = 7.4 Hz), 7.41 (2H, t, J = 7.4 Hz), 7.61 (2H, d, J = 7.4 Hz), 7.77 (2H, d, J = 7.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.1 (2C), 19.7, 22.7 (2C), 23.9, 24.8, 27.2 (2C), 29.08, 29.13, 29.2, 29.3 (2C), 29.35, 29.37, 29.54, 29.61, 29.67, 29.69, 29.70, 29.72, 29.73 (2C), 29.74 (2C), 29.8, 31.9 (2C), 34.2, 41.3, 47.1, 62.1 (d, J = 4.9 Hz), 66.0 (d, J = 5.8 Hz), 66.9, 67.5 (d, J = 6.3 Hz), 69.1 (d, J = 2.7 Hz), 70.2 (d, J = 5.8 Hz), 108.7, 116.3, 120.0 (2C), 125.1 (2C), 127.1 (2C), 127.7 (2C), 129.7, 130.0, 141.3 (2C), 143.8 (2C), 144.3, 156.4, 173.0; HRMS (FAB): m/z calcd for $C_{57}H_{89}N_2NaO_9P$, 999.6198; found, 999.6206 ([M + Na]$^+$).

(2R)-1-(((2-Aminoethoxy) (hydroxy)phosphoryl)oxy)-3-(((Z)-hexadec-1-en-1-yl)oxy)propan-2-yl oleate: PlsEtn-[16:0/18:1 n-9] (1)

To a stirred solution of **13** (40 mg, 0.041 mmol) in dry DMF (0.8 mL) was added piperidine (32 µL, 0.33 mmol) at 0 °C and the resulting mixture was warmed to ambient temperature. After stirring overnight, the reaction mixture was quenched with phosphate buffer (phosphate-buffered saline/saturated NaCl, pH = 7.2-7.4, 20 mL) and extracted 3 times with CH_2Cl_2. The extract was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was roughly purified by Cosmosil 75C_{18} −PREP ($CHCl_3$/MeOH = 1:4) to give crude **1**, which was purified by HPLC (COSMOSIL 5C_{18}-MS-II, 10 mm × 250 mm, 40 °C, UV: 210 nm, flow: 5 mL/min, MeOH) to give **1** (15.2 mg, 53%) as a pale yellow oil. $[\alpha]^{27}_D$ −0.47 (c 0.275, $CHCl_3$); IR: ν_{max} 3414 (br), 3006 (w), 2853 (m), 1738 (m), 1665 (m), 1466 (m), 1231 (m), 1080 (m); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.88 (6H, t, J = 7.2 Hz), 1.25-1.30 (44H, m), 1.57-1.63 (2H, m), 1.96-2.01 (6H, m), 2.32 (2H, t, J = 7.5 Hz), 3.13 (2H, brs), 3.84-4.00 (4H, m), 4.07 (2H, brs), 4.34 (1H, q, J = 10.7 Hz), 5.10-5.19 (1H, m), 5.30-5.38 (2H, m), 5.90 (1H, d, J = 6.2 Hz), 8.47 (2H, br); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.1 (2C), 22.70, 22.71, 23.9, 25.0, 27.2 (2C), 29.18, 29.24, 29.33 (2C), 29.34 (2C), 29.42 (2C), 29.44, 29.56, 29.71 (2C), 29.72, 29.77, 29.78, 29.80, 29.81, 29.82, 31.9, 32.0, 34.3, 40.4, 62.2, 63.8, 70.1, 71.4 (d, J = 7.5 Hz), 107.8, 129.7, 130.0, 144.7, 173.2; ^{31}P NMR (320 MHz, $CDCl_3$): δ 0.382; HRMS (FAB): m/z calcd for $C_{39}H_{77}NO_7P$, 702.5432; found, 702.5443 ($[M + H]^+$).

Supplementary material

Supplementary material is available at *Bioscience, Biotechnology, and Biochemistry* online.

Acknowledgments

We are grateful to Yuka Taguchi (Tohoku University) for NMR and MS measurements.

Data availability

The authors confirm that the data underlying this article are available in the article and in its online supplementary material.

Author contribution

Y.O. and S.K. designed the synthetic route. Y.O., K.N., Y.O., and S.K. wrote the manuscript. S.M., T.M., T.I., and Y.A. conducted the synthetic experiments with the aid of M.E. and Y.O.

Funding

None declared.

Disclosure statement

The authors declare no conflicts of interest associated with this manuscript.

References

Blank ML, Wykle RL, Snyder F. Enzymic synthesis of ethanolamine plasmalogens from an O-alkyl glycerolipid. *FEBS Lett* 1971;**18**:92-4.

Braverman NE, Moser AB. Functions of plasmalogen lipids in health and disease. *Biochim Biophys Acta* 2012;**1822**:1442-52.

Brites P, Waterham HR, Wanders RJ. Functions and biosynthesis of plasmalogens in health and disease. *Biochim Biophys Acta* 2004;**1636**:219-31.

Engelmann E. Plasmalogens: targets for oxidants and major lipophilic antioxidants. *Biochem Soc Trans* 2004;**32**:147-50.

Farooqui AA, Horrockes LA. Plasmalgen-selective phospholipase A_2 and its involvement in Alzheimer's disease. *Biochem Soc Trans* 1998;**26**:243-6.

Farooqui AA, Yang HC, Horrockes AL. Plasmalogens, phospholipases A_2 and signal transduction. *Brain Res Rev* 1995;**21**:152-61.

Fonteb AN, Chilton FH. Rapid remodeling of arachidonate from phosphatidylcholine to phosphatidylethanolamine pools during mast cell activation. *J Immunol* 1992;**148**:1784-91.

Ford DA, Gross RW. Plasmenylethanolamine is the major storage depot for arachitonic acid in rabbit vascular smooth muscle and is rapidly hydrolyzed after angiotensin II stimulation. *Proc Natl Acad Sci USA* 1989;**86**:3479-83.

Ginsberg L, Xuereb JH, Gershfeld NL. Membrane instability, plasmalogen content, and Alzheimer's disease. *J Neurochem* 1998;**70**:2533-8.

Glaser PE, Gross RW. Plasmenylethanolamine facilitates rapid membrane fusion: a stopped-flow kinetic investigation correlating the propensity of a major plasma membrane constituent to adopt an H_{II} phase with its ability to promote membrane fusion. *Biochemistry* 1994;**33**:5805-12.

Goldfine H. The appearance, disappearance and reappearance of plasmalogens in evolution. *Prog Lipid Res* 2010;**49**:493-8.

Goodenowe DB, Cook LL, Liu J *et al*. Peripheral ethanolamine plasmalogen deficiency: a logical causative factor in Alzheimer's disease and dementia. *J Lipid Res* 2007;**48**:2485-9.

Gross RW. High plasmalogen and arachidonic acid content of canine myocardial sarcolemma: a fast atom bombardment mass spectroscopic and gas chromatography-mass spectroscopic characterization. *Biochemistry* 1984;**23**:158-65.

Gross RW. Identification of plasmalogen as the major phospholipid constituent of cardiac sarcoplasmic reticulum. *Biochemistry* 1985;**24**:1662-8.

Hermetter A, Paltauf F. Semisynthetic preparation of choline and ethanolamine plasmalogens. *Chem Phys Lipids* 1982;**30**:47-53.

Hermetter A, Rainer B, Ivessa E *et al*. Influence of plasmalogen deficiency on membrane fluidity of human skin fibroblasts: a fluorescence anisotropy study. *Biochim Biophys Acta* 1989;**978**:151-7.

Horrocks LA, Sharma M. *Plasmalogens and O-alkyl glycerophospholipids*. In: Hawthome JN, Ansell GB (eds). *Phospholipids*. Amsterdam: Elsevier Biomedical Press, 1982, 51-93.

Khan MA, Wood PL, Goodenowe D. *Methods for the synthesis of plasmalogens and plasmalogen derivatives, and therapeutic uses thereof*. US Patent 2013071418, 2015.

Klenk E, Debuch H. Plasmalogens. In: Holman RT, Lundberg WO, Malkin T (eds). *Progress in the Chemistry of Fats and Other Lipids*. New York: Pergamon, 1963, 1-29.

McHowat J, Liu S, Creer MH. Selective hydrolysis of plasmalogen phospholipids by Ca^{2+}-independent PLA_2 in hypoxic ventricular myocytes. *Am J Physiol* 1998;**274**:C1727-37.

Mikkilineni AB, Kumar P, Abushanab E. The chemistry of L-ascorbic and D-isoascorbic acids. 2. *R* and *S* glyceraldehydes from a common intermediate. *J Org Chem* 1988;**53**:6005-9.

Morandat S, Bortolato M, Anker G *et al*. Plasmalogens protect unsaturated lipids against UV-induced oxidation in monolayer. *Biochim Biophys Acta* 2003;**1616**:137-46.

Murphy RC. Free-radical-induced oxidation of arachidonoyl plasmalogen phospholipids: antioxidant mechanism and precursor pathway for bioactive eicosanoids. *Chem Res Toxicol* 2001;**14**:463-72.

Nagan N, Zeoller RA. Plasmalogens: biosynthesis and functions. *Prog Lipid Res* 2001;**40**:199-229.

Oma S, Mawatari S, Saito K *et al*. Changes in phospholipid composition of erythrocyte membrane in Alzheimer's disease. *Dement Geriatr Cogn Dis Extra* 2012;**2**:298-303.

Otoki Y, Nakagawa K, Kato S *et al*. MS/MS and LC-MS/MS analysis of chloine/ethanol amine plasmalogens via promotion of alkali metal adduct formation. *J Chromatogr B* 2015;**1004**: 85-92.

Paltauf F, Holasek A. Enzymatic synthesis of plasmalogens. *J Biol Chem* 1973;**248**:1609-15.

Pfaendler HR, Weimar V. Synthesis of racemic ethanolamine plasmalogen. *Synthesis* 1996;**1996**:1345-9.

Portilla D, Creer MH. Plasmalogen phospholipid hydrolysis during hypoxic injury of rabbit proximal tubules. *Kidney Int* 1995;**47**:1087-94.

Qin D, Byun HS, Bittman R. Synthesis of plasmalogen via 2,3-bis-*O*-(4′-methoxybenzyl)-*sn*-glycerol. *J Am Chem Soc* 1999;**121**:662-8.

Reiss D, Beyer K, Engelmann B. Delayed oxidative degradation of polyunsaturated diacyl phospholipids in the presence of plasmalogen phospholipids in vitro. *Biochem J* 1997;**323**: 807-14.

Rui Y, Thompson DH. Efficient stereoselective synthesis of plasmenylcholines. *Chem Eur J* 1996;**2**:1505-8.

Shin J, Gerasimov O, Thompson DH. Facile synthesis of plasmalogens via barbier-type reactions of vinyl dioxanes and vinyl dioxanes with alkyl halides in LiDBB solution. *J Org Chem* 2002;**67**:6503-8.

Shin J, Qualls MM, Boomer JA *et al*. An efficient new route to plasmehyl-type lipids: synthesis and cytotoxicity of a plasmenylcholine analogue of the antitumor ether lipid ET-OMe. *J Am Chem Soc* 2001;**123**:508-9.

Shin J, Thompson DH. Direct synthesis of plasmenylcholine from ally-substituted glycerols. *J Org Chem* 2003;**68**:6760-6.

Snipes J, Suter U. *Its functions and metabolism in biology and medicine: subcellular biochemistry*. In: Bittman R (ed). *Cholesterol*. New York: Plenum Press, 1997, 173-204.

Snyder F, Blank ML, Wykle RL. The enzymic synthesis of ethanolamine plasmalogens. *J Biol Chem* 1971;**246**:3639-45.

Snyder F, Lee TC, Blank ML. *Plasmalogens: their metabolism and central role in the production of lipid mediators*. In: Gross RW (ed). *Advances in Lipobiology*. Stanford, CT: JAI Press, 1997, 261-86.

Sugiura T, Waku K. *Composition of alkyl ether-linked phospholipids in mammalian tissues*. In: Snyder F (ed). *Platelet Activating Factor and Related Lipid Mediators*. New York: Plenum Press, 1987, 55-85.

Swarts BM, Guo Z. Synthesis of a glycosylphosphatidylinositol anchor bearing unsaturated lipid chains. *J Am Chem Soc* 2010;**132**:6648-50.

Thompson DH, Gerasimov OV, Wheeler JJ *et al*. Triggerable plasmalogen liposomes: improvement of system efficiency. *Biochim Biophys Acta* 1996;**1279**:25-34.

Van den Bossche J, Shin J, Thompson DH. Improved plasmalogen synthesis using organobarium intermediates. *J Org Chem* 2007;**72**:5005-7.

Wood PL, Mankidy R, Ritchie S *et al*. Circulating plasmalogen levels and Alzheimer's disease assessment scale-cognitive scores in Alzheimer patients. *J Psychiatry Neurosci* 2010;**35**: 59-62.

Yamashita S, Kanno S, Honjo A *et al*. Analysis of plasmalogen species in foodstuffs. *Lipids* 2016;**51**:199-210.

Yamashita S, Kiko T, Fujiwara H *et al*. Alterations in the levels of amyloid-β, phospholipid hydroperoxide, and plasmalogen in the blood of patients with Alzheimer's disease: possible interactions between amyloid-β and these lipids. *J Alzheimers Dis* 2015;**50**:527-37.

Zoeller RA, Morand OH, Raetz CR. A possible role of plasmalogens in protecting animal cells against photosensitized killing. *J Biol Chem* 1988;**263**:11590-6.