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Synthesis and Antioxidant Properties of an Unnatural Plasmalogen Analogue Bearing a trans *O*-Vinyl Ether Linkage

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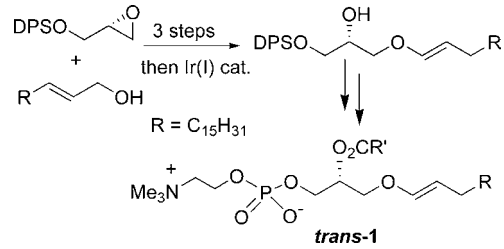
ABSTRACT



To assess the antioxidant behavior of *trans*-1, we first synthesized *trans*-allyl ether 4 by opening an (*S*)-glycidol derivative with an (*E*)-alk-2-en-ol, and then produced the unnatural E-enol ether 1 by a stereoselective iridium(I)-catalyzed olefin isomerization. Natural *cis*-1 was preferentially degraded by HOCl and was more protective than *trans*-1 against lipid peroxidation induced by a free-radical initiator, demonstrating that the geometry of the 1′-alkenyloxy bond participates in the antioxidant defensive role of 1.

Plasmalogens are a subclass of glycerophospholipids that bear an 1-*O*-alk-1′-(*Z*)-enyl chain at the *sn*-1 position of glycerol (usual chain length, C16 or C18), *sn*-2 fatty acyl chain (typically unsaturated or polyunsaturated), and a polar headgroup at the *sn*-3 position (predominantly a mixture of phosphoethanolamine and phosphocholine).[1] Plasmalogens comprise about 20% of the total phospholipids in humans; they are especially abundant in human cardiac muscle, skeletal muscle, and nervous tissues, which contain high levels of polyunsaturated fatty acids.[2] Since plasmalogen-deficient cells are more susceptible to free radical mediated oxidative damage than cells with normal plasmalogen levels,[3] and because enol ethers are known to be susceptible to oxidation,[4] it has been proposed that the *Z*-enol ether functionality, which is unique to plasmalogens, may serve as a trap for reactive oxygen species. The vinyl ether linkage is located in close proximity to the lipid−water interface, and may thus protect the polyunsaturated chains of phospholipids in membranes and lipoproteins from oxidation.[5] The putative intermediates formed by [2 + 2] and [2 + 4]

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(1) (a) Nagan, N.; Zoeller, R. A. *Prog. Lipid Res.* **2001**, *40*, 199–229. (b) Brites, P.; Waterham, H. R.; Wanders, R. J. *Biochim. Biophys. Acta* **2004**, *1636*, 219–231.

(2) Horrocks, L. A.; Sharma, M. In *Phospholipids*; Hawthorne, J. N., Ansell, G. B., Eds.; Elsevier: Amsterdam, 1982; pp 51−93.

(3) (a) Morand, O. H.; Zoeller, R. A.; Raetz, C. R. H. *J. Biol. Chem.* **1988**, *263*, 11597–11606. (b) Hoefler, G.; Paschke, E.; Hoefler, S.; Moser, A. B.; Moser, H. W. *J. Clin. Invest.* **1991**, *88*, 1873–1879.

(4) For examples of 1,2-dioxetane formation from the reaction of enol ethers with singlet oxygen, see: Gollnick, K.; Knutzen-Mies, K. *J. Org. Chem.* **1991**, *56*, 4017–4027.

(5) (a) Engelmann, B.; Bräutigam, C.; Thiery, J. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 1235–1242. (b) Reiss, D.; Beyer, K.; Engelmann, B. *Biochem. J.* **1997**, *323*, 807–814. (c) Hahnel, D.; Beyer, K.; Engelmann, B. *Free Radical Biol. Med.* **1999**, *27*, 1087–1094.

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cycloaddition of singlet oxygen to this linkage are dioxetane and ene intermediates, respectively, which subsequently undergo degradation to generate a complex mixture of fatty aldehydes (such as pentadecanal), 1-formyl-2-acyl-*sn*-glycerophospholipids, and 1-(*O*-1′-hydroperoxy)-2-acyl-*sn*-glycero-3-phospholipids.[6] Decomposition of the allylic hydroperoxides produces plasmalogen epoxides via a radical process, and 1-formyl- and 1-lyso-phospholipids by hydrolytic processes.[6d,7]

Plasmalogens are more susceptible to oxidation than phosphatidylcholine (PC) and sphingomyelin, the other major membrane phospholipids;[8] however, sphingomyelin has also been found to inhibit PC peroxidation, albeit less efficiently than plasmalogen, but its long-chain base contains a trans double bond near the membrane-water interface.[9a] To elucidate the role of the naturally occurring (*Z*)-*O*-vinyl linkage of plasmalogen in protecting polyunsaturated lipids from oxidative degradation, we describe herein the first chemical synthesis of an unnatural analogue of plasmalogen with an (*E*)-*O*-vinyl linkage at the *sn*-1 position (*trans*-**1**, Figure 1)[10] and a comparison of the antioxidant effects of *cis*- and *trans*-**1** in two model systems.

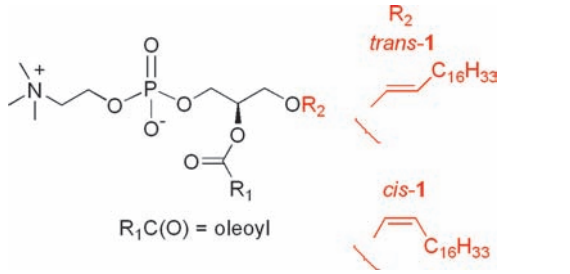


Figure 1. Structures of naturally occurring *cis*-**1** and the unnatural *trans*-**1** analogue.

Hypochlorous acid is a highly reactive oxidant and chlorinating agent that is produced endogenously when physiological concentrations of chloride ion are oxidized by the myeloperoxidase-catalyzed decomposition of hydrogen peroxide.[11] HOCl reacts with many biological molecules, including the double bonds of lipids, to generate chlorinated products.[13] Recently, Davies and co-workers showed that the kinetics of vinyl ether oxidation is several orders of magnitude greater than that of aliphatic alkenes.[14] The products of plasmalogen oxidation by HOCl are 2-chloro fatty aldehydes and 1-lysophosphatidylcholine (LPC).[15] Phospholipid chlorohydrins in the *sn*-2 chain of unsaturated LPC molecular species are also formed as secondary reaction products by electrophilic attack of HOCl on alkenyl double bonds.[16] These chlorinated lipid species accumulate in activated neutrophils, monocytes, ischemic myocardium, and human atherosclerotic lesions and have potential roles in many inflammatory disorders.[17]

Peroxidation of phospholipids containing a polyunsaturated fatty acyl chain such as linolenic acid has been studied frequently by using free-radical initiators, such as the water-soluble thermolabile free-radical generator 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH).[18] As a model system to compare the antioxidant capability of *cis*- and *trans*-**1**, we studied the influence of **1** on the rate and extent of peroxidation of 1-palmitoyl-2-linolenoylphosphatidylcholine (16:0−18:2-PC) in liposomes exposed to AAPH. The reaction kinetics were monitored by measuring the formation of conjugated diene lipid hydroperoxides, as monitored by the absorbance at 234 nm.[9] A_{234} is diminished when AAPH-induced free-radical chain initiation in the polyunsaturated acyl chain is suppressed.

Previous reports of the preparation of 1-*O*-alkenyl ether derivatives of glycerol were based on elimination reactions of α-halo cyclic glycerol acetals, which gave low yields and poor stereoselectivity.[19] To construct the glycerol backbone with an *O*-1′-alkenyl chain at the *sn*-1 position, we used (*S*)-glycidol and 1-hexadecanol as the starting materials for the synthesis of *trans*-**1** (see Supporting Information). As shown in Scheme 1, the DPS ether of (*S*)-glycidol (**3**) was employed in a regioselective BF_3•OEt_2-mediated ring-opening reaction[20] of (*E*)-octadec-2-en-1-ol (**2**), prepared by DIBALH reduction of ethyl (*E*)-octadecanoate. The expected attack at C-3

(6) (a) Zoeller, R. A.; Lake, A. C.; Nagan, N.; Gaposchkin, D. P.; Legner, M. A.; Lieberthal, W. *Biochem. J.* **1999**, *338*, 769–776. (b) Zoeller, R. A.; Morand, O. H.; Raetz, C. R. H. *J. Biol. Chem.* **1988**, *263*, 11590–11596. (c) Stadelmann-Ingrand, S.; Favreliere, S.; Fauconneau, B.; Mauco, G.; Tallineau, C. *Free Radical Biol. Med.* **2001**, *31*, 1263–1271. (d) Thompson, D. H.; Inerowicz, H. D.; Grive, J.; Sarna, T. *Photochem. Photobiol.* **2003**, *78*, 323–330.

(7) Loidl-Stahlhofen, A.; Hannemann, K.; Felde, R.; Spiteller, G. *Biochem. J.* **1995**, *309*, 807–812.

(8) Hofer, G.; Lichtenberg, D.; Kostner, G. M.; Hermetter, A. *Clin. Biochem.* **1996**, *29*, 445–450.

(9) (a) Subbaiah, P. V.; Sircar, D.; Lankalapalli, R. S.; Bittman, R. *Arch. Biochem. Biophys.* **2009**, *481*, 72–79. (b) Recknagel, R. O.; Glende, E. A. Jr. In *Methods Enzymol.*, Vol. 105; Packer, L., Ed. Academic: San Diego 1984, pp. 331−337.

(10) A semisynthetic route to racemic *trans*-**1** via a lipase-catalyzed hydrolysis of an 1-(alkenyloxy)-2,3-diacylglycerol was reported: Slotboom, A. J.; De Haas, G. H.; van Deenen, L. L. M. *Chem. Phys. Lipids* **1967**, *1*, 192–208.

(11) Senthilmohan, R.; Kettle, A. J. *Arch. Biochem. Biophys.* **2006**, *445*, 235–244.

(12) Pattison, D. I.; Davies, M. J. *Curr. Med. Chem.* **2006**, *13*, 3271–3290.

(13) Albert, C. J.; Crowley, J. R.; Hsu, F. F.; Thukkani, A. K.; Ford, D. A. *J. Biol. Chem.* **2001**, *276*, 23733–23741.

(14) Skaff, O.; Pattison, D. I.; Davies, M. J. *Biochemistry* **2008**, *47*, 8237–8245.

(15) Messner, M. C.; Albert, C. J.; Hsu, F. F.; Ford, D. A. *Chem. Phys. Lipids* **2006**, *144*, 34–44. Primary amines react with HOCl to give chloramines; therefore, we synthesized **1** with a phosphocholine instead of a phosphoethanolamine head group to target the *O*-vinyl ether linkage in the HOCl reaction and avoid having a competing reactive site.

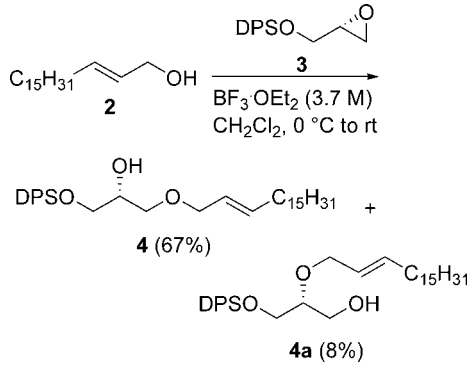
(16) (a) Thukkani, A. K.; Albert, C. J.; Wildsmith, K. R.; Messner, M. C.; Martinson, B. D.; Hsu, F. F.; Ford, D. A. *J. Biol. Chem.* **2003**, *278*, 36365–36372. (b) Thukkani, A. K.; Hsu, F. F.; Crowley, J. R.; Wysolmerski, R. B.; Albert, C. J.; Ford, D. A. *J. Biol. Chem.* **2002**, *277*, 3842–3849.

(17) (a) Thukkani, A. K.; Martinson, B. D.; Albert, C. J.; Vogler, G. A.; Ford, D. A. *Am. J. Physiol. Heart Circ. Physiol.* **2005**, *288*, H2955–H2964. (b) Thukkani, A. K.; McHowat, J.; Hsu, F. F.; Brennan, M. L.; Hazen, S. L.; Ford, D. A. *Circulation* **2003**, *108*, 3128–3133. (c) Spickett, C. M. *Pharmacol Ther.* **2007**, *115*, 400–409.

(18) Niki, E. In *Methods Enzymol.*, Vol. 186; Packer, L., Glazer, A. N., Eds.; Academic: San Diego, 1990; pp 100−108.

(19) For syntheses of mixtures of 1-*O*-(1′-alkenyl)glycerols and 2-*O*-(1′-alkenyl)glycerols containing E/Z mixtures, see: (a) Piantadosi, C. F.; Hirsch, A. F.; Yarbro, C. L.; Anderson, C. E. *J. Org. Chem.* **1963**, *28*, 2425–2428. (b) Craig, J. C.; Hamon, D. P. G. *J. Org. Chem.* **1965**, *30*, 4168–4175. (c) Otera, J.; Niibo, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3977–3978. (d) Pfaendler, H. R.; Müller, F. X. *Synthesis* **1992**, 350–352.

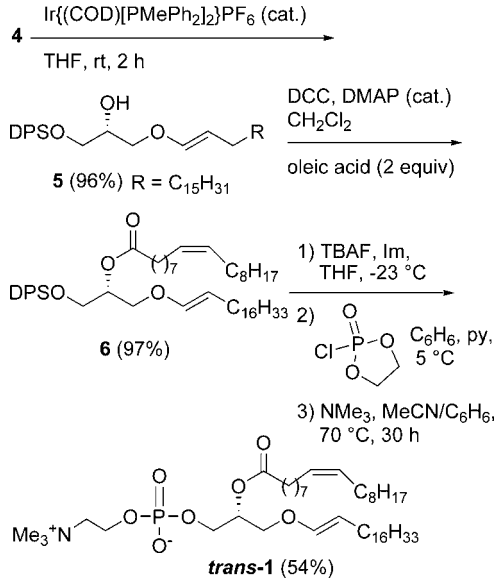
Scheme 1. Preparation of Allyl Ether **4**



provided an 8:1 (separable) mixture of allyl ether **4** and the undesired regioisomer **4a**.

Next, isomerization of the double bond was investigated using transition metal complexes as catalysts.[21] An initial attempt using $RhCl_3{\cdot}3H_2O$[22] as a catalyst resulted in no reaction. Fortunately, we found that use of (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (Ir(COD)[$PMePh_2$]$_2PF_6$),[23] after activation with hydrogen for 5 min, resulted in the stereoselective isomerization of allyl ether **4** to enol ether **5** in 2 h at rt, and with the desired E double bond as the only product (Scheme 2);

Scheme 2. Ir(I)-Mediated Olefin Isomerization and Final Conversion to *trans*-**1**



$J_{H1'\text{-}H2'}$ = 12.6 Hz at δ 6.24 ppm in the ^{1}H NMR spectrum of **5** is indicative of the desired E isomer, which was obtained in 96% yield.[24] *sec*-Alcohol **5** was esterified with oleic acid, using DCC in the presence of DMAP, affording ester **6**. The final steps in the synthesis of *trans*-**1** required conditions that did not cleave the acid-labile and oxidizable vinyl ether moiety or the alkaline-labile acyl ester bond. After the silyl ether in **6** was removed using TBAF (6 equiv) and imidazole (2.5 equiv) in THF at −23 °C, the phosphocholine headgroup was installed at the *sn*-3 position in 54% overall yield without accompanying acyl migration by opening of a cyclic phospholane intermediate in a pressure tube with anhydrous Me_3N (condensed at −10 °C) in MeCN/C_6H_6 (3:1) in the presence of pyridine.[25]

The first model system we investigated was the reaction of *cis*- and *trans*-**1** with HOCl. The HOCl oxidation reaction of *cis*- and *trans*-**1** was analyzed using increasing concentrations of HOCl (Figure 2). At both a 2- and 5-fold molar ratio of [HOCl]/[**1**], the cis-vinyl ether linkage was oxidized faster than the trans linkage. This selectivity was not observed

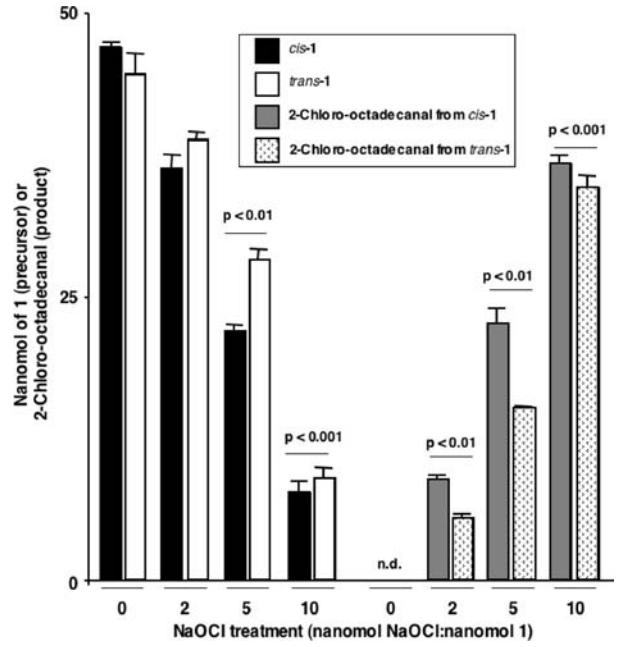


Figure 2. Disparate *cis*- and *trans*-**1** oxidation by HOCl. The bars show the amounts in nmol of **1** remaining (left panel) and 2-chlorooctadecanal produced (right panel) after treatment with various HOCl/**1** molar ratios for 5 min at 37 °C. Data are the mean ± SD of 3 experiments. See Supporting Information for experimental details.

(20) For $BF_3{\cdot}OEt_2$-mediated opening of glycidyl derivatives with saturated aliphatic alcohols, see: (a) Guivisdalsky, P. N.; Bittman, R. *J. Am. Chem. Soc.* **1989**, *111*, 3077–3079. (b) Guivisdalsky, P. N.; Bittman, R. *J. Org. Chem.* **1989**, *54*, 4637–4642.

(21) (a) Krompiec, S.; Kuźnik, N.; Urbala, M.; Rzepa, J. *J. Mol. Catal. A: Chem.* **2006**, *248*, 198–209. (b) Kuźnik, N.; Krompiec, S. *Coord. Chem. Rev.* **2007**, *251*, 222–233.

(22) Grieco, P. A.; Nishizawa, M.; Marinovic, N.; Ehmann, W. J. *J. Am. Chem. Soc.* **1976**, *98*, 7102–7104.

(23) (a) Oltvoort, J. J.; Van Boeckel, C. A. A.; De Koning, J. H.; Van Boom, J. H. *Synthesis* **1981**, 305–308. (b) Westcott, S. A. In *e-EROS Encyclopedia of Reagents for Organic Synthesis*; Wiley: Chichester, UK, 2001.

(24) The Z isomer of **5** was prepared by Lindlar reduction of an *O*-alkynyl ether; for the high-field doublet of the cis-*O*-vinyl proton $J_{H1'\text{-}H2'}$ = 6.20 Hz at δ 5.94 ppm.[25]

(25) Qin, D. H.; Byun, H. S.; Bittman, R. *J. Am. Chem. Soc.* **1999**, *121*, 662–668.

at a higher [HOCl]/[**1**] molar ratio, probably because competing reactions that consume HOCl take place, such as addition to the double bond in the *sn*-2 fatty acyl chain of **1**.[17c]

ESI-MS analyses of the reaction mixture with 1 mM HOCl demonstrated that the other product of HOCl oxidation is LPC containing an oleic acid moiety (*m*/*z* 544.24) (Figure 3). The chlorohydrin of this material, formed by reaction with HOCl,[15] is a byproduct with *m*/*z* 596.18.

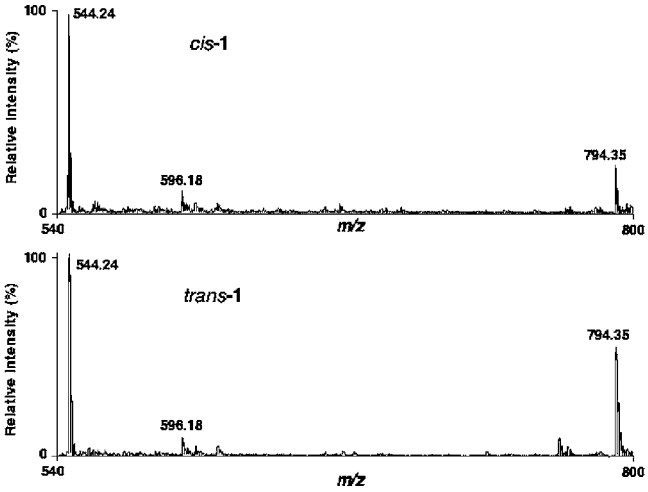


Figure 3. ESI-MS of the HOCl oxidation products. *cis*- or *trans*-**1** (50 nmol; $(M + Na)^+$, *m*/*z* 794.35) was incubated in 500 μL of 20 mM phosphate-buffered saline containing 0.1 mM diethylenetriaminepentaacetic acid (pH 7.0) in the presence of 1 mM chloride-free NaOCl for 5 min at 37 °C.[26]

Plasmalogens are known to protect membrane phospholipids against radical-induced damage.[1a] Therefore, we next evaluated the ability of *cis*- and *trans*-**1** to protect 16:0−18:2-PC from AAPH (3.3 mM) induced peroxidation in liposomes prepared with 80 mol % 16:0−18:2-PC and 20 mol % *cis*- or *trans*-**1**. The oxidation of 16:0−18:2-PC was monitored by the increase in A_{234},[27] a measure of conjugated diene hydroperoxide formation in the *sn*-2 fatty acyl group of PC.[28] *cis*-**1** inhibited the rate of 16:0−18:2-PC oxidation by 33% vs only 12% inhibition by *trans*-**1**, based on the slopes of the reaction curves in the linear range (0−100 min; Figure 4). Similar data were obtained when 1 mM AAPH was used (data not shown). We also determined the effect of *cis*- and *trans*-**1** on 16:0−18:2-PC oxidation in liposomes in the presence of 50 μM Cu^{2+} as the oxidizing agent, which facilitates free-radical chain propagation.[29] We found that *trans*-**1** was completely ineffective in protecting 16:0−18:2-PC oxidation whereas *cis*-**1** inhibited PC oxidation by 45% (results not shown).

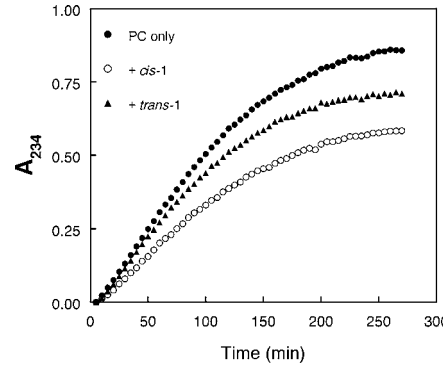


Figure 4. Effect of *cis*- and *trans*-**1** on the oxidation of 16:0−18:2-PC induced by AAPH.

In summary, the first chemical synthesis of *trans*-**1**, an unnatural analogue of plasmalogen bearing a trans *O*-vinyl linkage at the *sn*-1 position, has been achieved. A key step involves E stereoselective enol ether formation of **5** via an iridium(I)-mediated olefin isomerization of *O*-allyl ether **4**. The data shown in Figures 2 and 4 indicate that the geometry of the alkenyl ether linkage of plasmalogen plays a significant role in plasmalogen's antioxidant properties.

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Supporting Information Available: Experimental procedures and NMR spectra for the reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) The reactions were terminated by addition of MeOH, the products were extracted into $CHCl_3$, resuspended in 500 μL of MeOH/$CHCl_3$ (4:1) containing 1 μM NaOH, and analyzed by ESI-MS (flow rate, 3 μL/min). Samples were analyzed in the positive ion mode with detection of their sodiated adducts.

(27) To correct for changes in A_{234} arising from AAPH-induced plasmalogen oxidation, liposomes were prepared with only *cis*- or *trans*-**1** at the same concentrations present in the 16:0−18:2-PC + **1** liposomes.

(28) Esterbauer, H.; Striegl, G.; Puhl, H.; Rotheneder, M. *Free Radical Res. Commun.* **1989**, *6*, 67–75.

(29) Burkitt, M. J. *Arch. Biochem. Biophys.* **2001**, *394*, 117–135.