

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

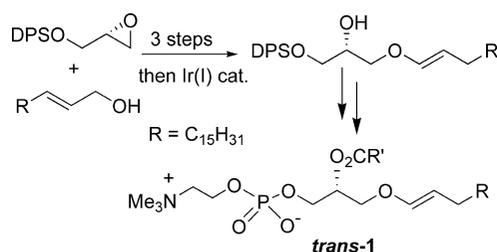
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ABSTRACT



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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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.

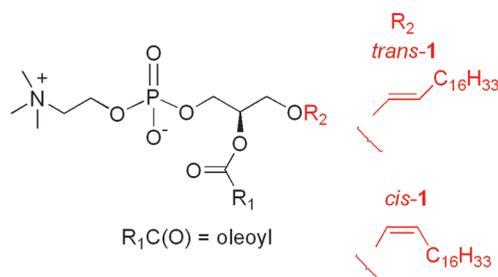


Figure 1. Structures of naturally occurring *cis*-1 and the unnatural *trans*-1 analogue.

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,¹²

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-linolenylphosphatidylcholine (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 A₂₃₄ 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 BF₃·OEt₂-mediated ring-opening reaction²⁰ of (*E*)-octadec-2-en-1-ol (**2**), prepared by DIBALH reduction of ethyl (*E*)-octadecanoate. The expected attack at C-3

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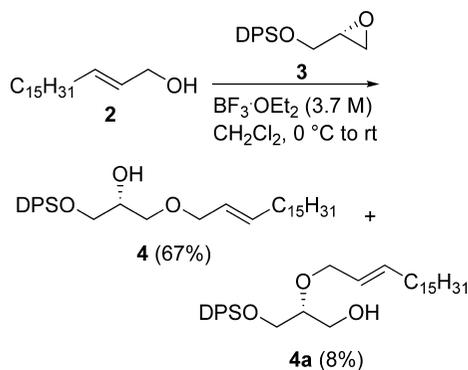
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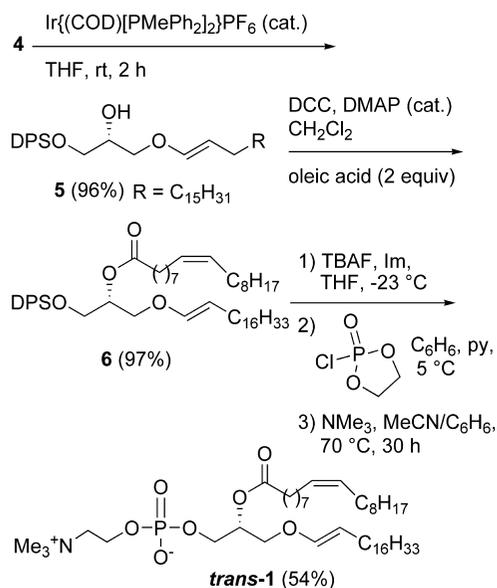
Scheme 1. Preparation of Allyl Ether **4**



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**



$J_{H1'-H2'} = 12.6$ Hz at δ 6.24 ppm in the 1H NMR spectrum of **5** is indicative of the desired E isomer, which was obtained

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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 $-23^\circ C$, 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 $-10^\circ C$) 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

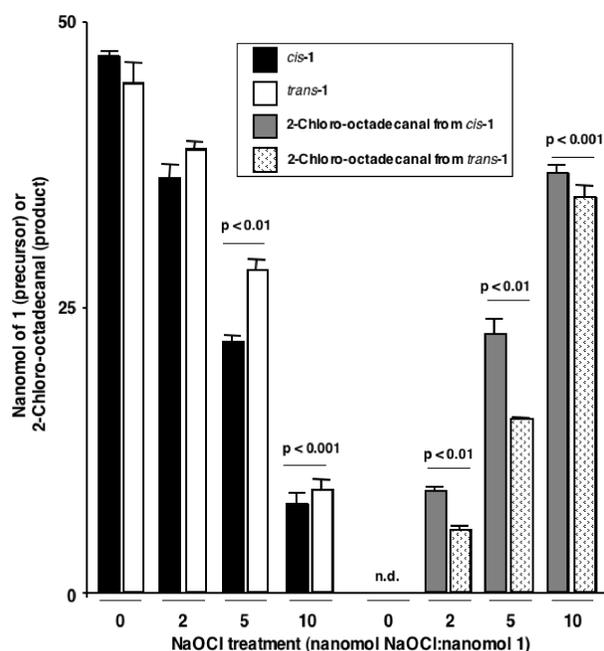


Figure 2. Disparate *cis*- and *trans*-**1** oxidation by HOCl. The bars show the amounts in nmol of **1** remaining (left panel) and 2-chlorooctadecanal produced (right panel) after treatment with various HOCl/**1** molar ratios for 5 min at $37^\circ C$. Data are the mean \pm SD of 3 experiments. See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

ratio of $[HOCl]/[1]$, the *cis*-vinyl ether linkage was oxidized faster than the *trans* linkage. This selectivity was not observed

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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.

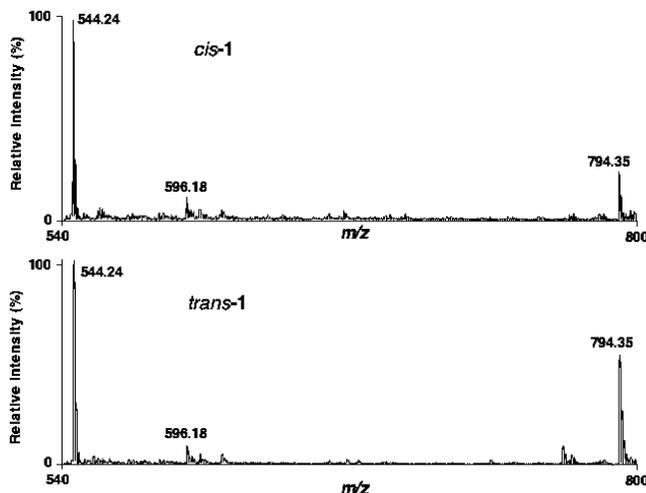


Figure 3. ESI-MS of the HOCl oxidation products. *cis*- or *trans*-**1** (50 nmol; (M + Na)⁺, *m/z* 794.35) was incubated in 500 μ L of 20 mM phosphate-buffered saline containing 0.1 mM diethylenetriaminepentaacetic acid (pH 7.0) in the presence of 1 mM chloride-free NaOCl for 5 min at 37 $^{\circ}$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

(26) The reactions were terminated by addition of MeOH, the products were extracted into CHCl₃, resuspended in 500 μ L of MeOH/CHCl₃ (4:1) containing 1 μ M NaOH, and analyzed by ESI-MS (flow rate, 3 μ L/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.

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

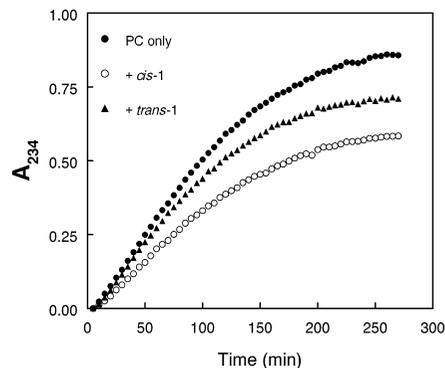


Figure 4. Effect of *cis*- and *trans*-**1** on the oxidation of 16:0–18:2-PC induced by AAPH.

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 μ M Cu²⁺ 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).

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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