An Efficient New Route to Plasmenyl-type Lipids: Synthesis and Cytotoxicity of a Plasmenylcholine Analogue of the Antitumor Ether Lipid ET-18-OMe

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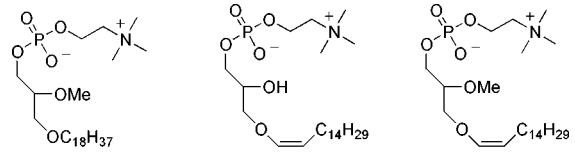
Plasmalogens[1−3] (i.e. plasmenylcholines or plasmenylethanolamines) are an arachidonate-rich family of mammalian phospholipids containing *sn*-1-*Z*-1′-*O*-alkenyl chains of varying length and degrees of unsaturation. They are members of a broad class of ether-linked phospholipids which also includes the cytotoxic antitumor ether lipids (ATL): single chain ether lipids that interfere with phospholipid metabolism and signal transduction pathways. One such ATL, 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OMe, edelfosine), induces apoptosis in a variety of Fas-positive tumor cell lines[4] and has shown promising activity in Phase II clinical trials for bone marrow purging autologous transplantation treatment of acute leukemia.[5]



ET-18-OMe LysoPlsCho PlsMeCho

The involvement of plasmalogens in cell signaling pathways, combined with their utility in drug and gene delivery applications,[6−8] has prompted the development of a more direct route to this important class of phospholipids. We now report the development of a facile new pathway to glyceryl vinyl ether lipids based on the addition of alkyllithium reagents to 2-vinyl-1,3-dioxolane (Figure 1) or 5-methoxy-2-vinyl-1,3-dioxane intermediates (Figure 2). This method has been applied to the synthesis of 1-*O*-1′-(*Z*)-hexadecenyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (PlsMeCho), a *Z*-vinyl ether analogue of ET-18-OMe, that shows significant antitumor activity in MIAPACA-2 pancreatic tumor cells.

Our initial investigations focused on Micheal-type additions of preformed alkyllithium reagents to 2-vinyl-1,3-dioxolane[9] as a model substrate (Figure 1) using lithium-iodide exchange,[10,11]

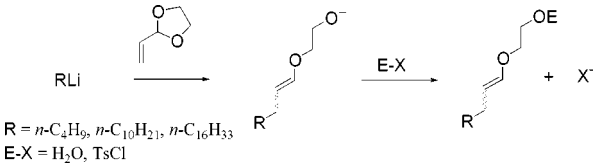


Figure 1. Generalized pathway for the preparation of vinyl ether lipids from 2-vinyl-1,3-dioxolane.

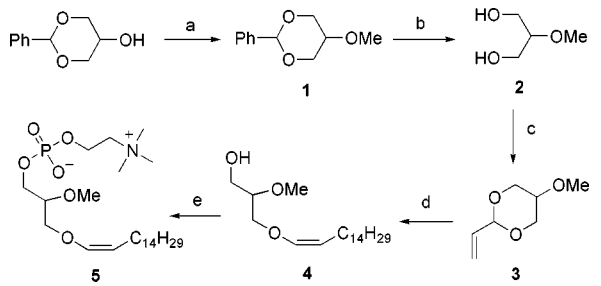


Figure 2. Pathway for the synthesis of 1-*O*-1′-(*Z*)-hexadecenyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (PlsMeCho): (a) NaH, MeI, THF, 25 °C, 3 h (92%); (b) AG 50W-X2 resin, THF, H_2O, reflux, 5 h (100%); (c) acrolein, *n*-$BuSnCl_3$, 25 °C, 1 h (28% trans, 36% cis); (d) Li, DBB, $C_{13}H_{27}I$, THF, 0 °C, 0.5 h (53%); (e) (*i*) 2-oxo-2-chloro-1,3,2-dioxaphospholane, Et_3N, C_6H_6, 5 °C, 20 h; (*ii*) Me_3N, MeCN/C_6H_6, 70 °C, 26 h (62% for steps *i* and *ii*, combined).

direct lithiation,[12] and lithiation catalyzed by 4,4-di-*tert*-butylbiphenyl (DBB).[13,14] Conditions for the reaction between 2-vinyl-1,3-dioxolane and the alkyllithium species were varied to determine the best conditions for *Z*-vinyl ether product formation (Table 1). Although couplings resulting from lithium−iodide exchange gave a modest excess of *Z*-vinyl ether product (60:40 *Z*:*E* ratio) in 51% yield, LiDBB-mediated reactions under Barbier-type conditions[15,16] were much more efficient, giving the corresponding vinyl ether adduct in 61% yield with *Z*:*E* ratios as high as 87:13.

On the basis of these observations, a related synthetic pathway for PlsMeCho was explored (Figure 2). This sequence begins with the preparation of 5-methoxy-2-vinyl-1,3-dioxane (**3**) via methylation of 2-phenyl-1,3-dioxan-5-ol, cleavage of acetal **1** under acidic conditions, and condensation of **2** with acrolein in the presence of *n*-$BuSnCl_3$.[17] The cis:trans ratio of **3** prepared in this manner was 1.3:1, as determined by ^{1}H NMR comparisons with *cis*- and *trans*-5-methyl-2-phenyl-1,3-dioxane.[18] The key step in this strategy, coupling of **3** under Barbier-type reaction conditions with 1-chloroalkanes, was then conducted in the presence of lithium 4,4-di-*tert*-butylbiphenyl radical anion (LiDBB). Reaction of *cis*-**3** and 1-chlorotridecane under Barbier conditions successfully gave the penultimate alcohol **4** in 49% yield and 88:12 *Z*:*E* ratio (Table 2).[19] Similar results were obtained by using *trans*-**3** as substrate. As the data in Table 2 also show, the highest yields of the desired **4**-(*Z*) product were obtained by using a 2-fold excess of 1-iodotridecane relative to **3** under Barbier conditions (53% yield, 95:5 *Z*:*E*). Introduction of the phosphocholine headgroup in the final step was accomplished by treating alcohol **4**-(*Z*) with

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(19) The stereochemistry of the adducts was established by ^{1}H NMR. *E* isomer: J = 13 Hz, 6.26 ppm (−O−CH═C), 4.80 ppm (−O−C═CH−). *Z* isomer: J = 7 Hz, 5.85 ppm (−O−CH═C), 4.43 ppm (−O−C═CH−).

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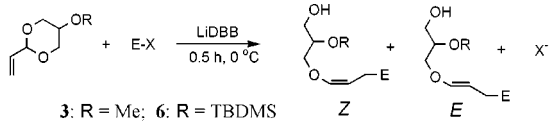
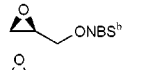
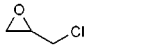
Table 1. Alkyllithium Additions to 2-Vinyl-1,3-dioxane

RLi	E−X	% yield	*Z*:*E*
n-BuLi	H_2O	43	40:60
n-$C_{10}H_{21}$I/*t*-BuLi	TsCl	51	60:40
n-$C_{13}H_{27}$Cl/Li	TsCl	36	31:69
n-$C_{13}H_{27}$Cl/LiDBB	TsCl	27	40:60
n-$C_{13}H_{27}$Cl/LiDBB (Barbier cond.)	TsCl	61	87:13

Table 2. Barbier-type Reactions of 2-Vinyl-1,3-dioxanes



3: R = Me; **6**: R = TBDMS *Z* *E*

Substrate	Electrophiles	equiv. E	% Yield	*Z*:*E*	Comments
trans-**3**	*n*-$C_{13}H_{27}$I	2	53	95:5	—
cis-**3**	*n*-$C_{13}H_{27}$Cl	0.5	49	88:12	—
trans-**6**	*n*-$C_{13}H_{27}$Cl	1	33	24:76	—
trans-**6**	*n*-$C_{13}H_{27}$Cl	2	57	30:70	—
trans-**6**	*n*-$C_{13}H_{27}$Cl	5	53	30:70	—
cis-**6**	*n*-$C_{13}H_{27}$Br	2	32	67:33	—
cis-**6**	*n*-$C_{13}H_{27}$I	2	47	98:2	—
cis-**6**	*n*-$C_{13}H_{27}$Ms[a]	2	47	0	 >98% cis
cis-**6**		2	0	0	
cis-**6**		2	41	0	 >98% cis

[a] Ms ≡ methanesulfonate. [b] NBS ≡ *m*-nitrobenzenesulfonate.

2-oxo-2-chloro-1,3,2-dioxaphospholane, followed by excess trimethylamine,[2,3] to give racemic PlsMeCho (**5**) in 62% yield.

The cytotoxicity of PlsMeCho was evaluated in MIAPACA-2 cells and compared to the cytotoxicities of ET-18-OMe, ET-16-OMe, and the corresponding semisynthetic lysoplasmenylcholine (LysoPlsCho)[20] (Figure 3) using the MTT assay. Cells were exposed to 10 μM lipid in Dulbecco's modified media for 24 h then examined for viability after incubation with MTT for 3−4 h. Our results indicate that PlsMeCho exhibits similar cytotoxicity within experimental error as the antitumor ether lipids ET-18-OMe and ET-16-OMe in MIAPACA-2 cells. These observations are consistent with the apoptosis of cells that actively accumulate ATL.[4,21−23] Originally, this sensitivity was thought to arise from the absence of *O*-alkyl cleavage enzyme activity;[24] however, it is now recognized that the cytotoxic and cytostatic properties of ATL are attributable to their interferences with both CTP:

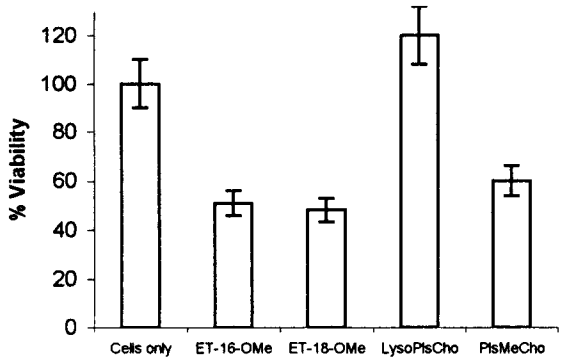


Figure 3. Viability of MIAPACA-2 cells after a 24 h exposure to 10 μM lipid concentrations as determined by MTT assay. Cells were plated at 15 000 cells/well (96 well plates) in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL). The reported data represent the mean and standard deviation of quadruplicate determinations.

phosphocholine cytidylyltransferase-mediated phosphatidylcholine synthesis[25,26] and cell cycling processes leading from G_0/G_1 to S phase.[27] They further suggest that the insensitivity of MIAPACA-2 cells to LysoPlsCho may be due to the rapid remodeling of this lysolipid precursor, via reacylation, after uptake. This would have the net effect of maintaining phospholipid homeostasis[28] while augmenting the cellular plasmalogen pool.

In conclusion, PlsMeCho has been prepared via a facile reaction sequence with use of an acrolein acetal derivative and 1-iodotridecane as precursors under Barbier-type reaction conditions. The observed regioselectivities and high Z:E ratios of the LiDBB-mediated coupling reaction are likely due to a lithiated allylic anion that undergoes stereoselective γ-coupling with electrophiles[29] via lithium ion−acetal chelation. The resulting racemic PlsMeCho shows cytotoxic properties similar to the clinically relevant ATL analogue ET-18-OMe; however, it is not presently known whether the cytotoxic mechanism for PlsMeCho is apoptotic in nature as it is for ET-18-OMe. Experiments designed to adapt this method for the preparation of plasmalogens and the pure *R*-**5**/*S*-**5** stereoisomers are in progress. Additional experiments aimed at probing the relative cytotoxicity of *R*-**5**, *S*-**5**, and racemic **5,** as well as the mechanism of cell death upon exposure to these ATL, are also planned.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectral data for compounds **1**−**5** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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