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Improved Plasmalogen Synthesis Using Organobarium Intermediates

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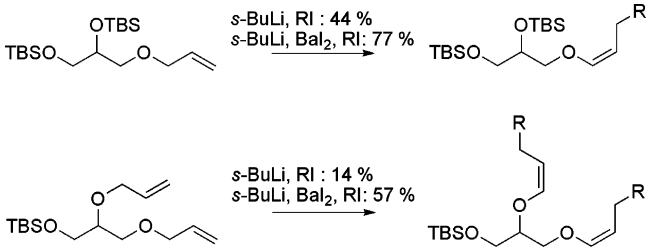

An improved synthesis of plasmalogen type lipids is described. Transmetalation of lithioalkoxy allyl intermediates with BaI_2 and subsequent alkylation with 1-iodoalkanes enables the stereoselective formation of *O*-(Z)-alkenyl ether as precursors for the synthesis of plasmenyl- and bisplasmenylcholines. This method provides a simple and adaptable approach for the stereocontrolled synthesis of plasmenyl derivatives with variations at the *sn*-1, *sn*-2, and *sn*-3 positions of the glycerol backbone.

Plasmalogens (i.e., plasmenylcholines or plasmenylethanolamines) are a class of naturally occurring phospholipids that are characterized by the presence of an *O*-(Z)-alkenyl ether at the *sn*-1 position. They are found predominantly within electrically active tissues such as brain, myelin, and heart. They account for 18% of the total human phospholipid mass.[1] Interest in the biochemical and biophysical properties of these unusual natural products has grown because of their role in many important biological processes such as signal transduction,[2] membrane fusion,[3,4] and lipid peroxidation.[5] The reactivity of these compounds toward both acidic conditions[6−10] and photooxidation[11,12] suggests that plasmalogen derivatives are promising candidates for the development of environmentally responsive gene[9] and drug delivery systems.[6−8,10−12] Since these lipids have not been isolated using conventional separation approaches,

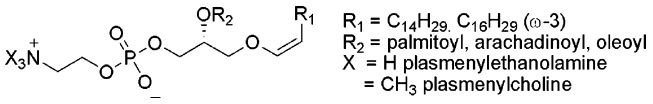

FIGURE 1. General structure of plasmalogen type lipids.

attention has shifted to synthetic methods as a means to obtain these compounds in pure form. The first efforts to synthesize members of this lipid class were made over 40 years ago.[13] The main challenge in the synthesis of plasmalogen derivatives is the stereoselective installation of the (*Z*)-enol ether linkage. Unfortunately, the sensitivity of the vinyl ether bond toward oxidants and acidic reagents places great limitations on the types of reagents and protective groups that can be employed in the synthetic pathway. The first stereoselective synthesis of plasmenylcholine, reported by Rui and Thompson,[14] involves the conversion of acyl glycerol derivatives to their corresponding vinyl phosphates, followed by palladium(0)-catalyzed reduction yielding the desired (*Z*)-enol ether. Other strategies that have been reported include Lindlar-catalyzed reduction of alkynyl ethers,[15] LiDBB-mediated reductive cleavage of acrolein acetal derivatives under Barbier conditions,[16] and more recently, the alkylation of lithioalkoxy allyl intermediates with alkyl iodides.[17] This note reports an improvement of the latter method and extends the methodology to the synthesis of bisplasmenylcholine using a TBS-protected 1,2-*O*-di(allyl)-glycerol precursor. Previous efforts have shown that the reaction of lithioalkoxyallyl intermediates with haloalkanes produces a mixture of α- and γ-coupled products, with a modest selectivity for the γ-substituted product (54−89% yield). This selectivity arises from the use of bulky allyl ether precursors and less hindered alkyl halides.[18,19] Shin and Thompson utilized these reagents in the synthesis of plasmenylcholines in moderate yield.[11] However, for the synthesis of bisplasmenylcholines, this procedure generates a complex mixture of products and very low yields of the desired bisvinyl ether species. Yanagisawa et al. have reported that allylbarium reagents can greatly improve both the yield (99%) and regioselectivity (99:1) of alkylations with alkyl halides when compared to the corresponding allyllithium

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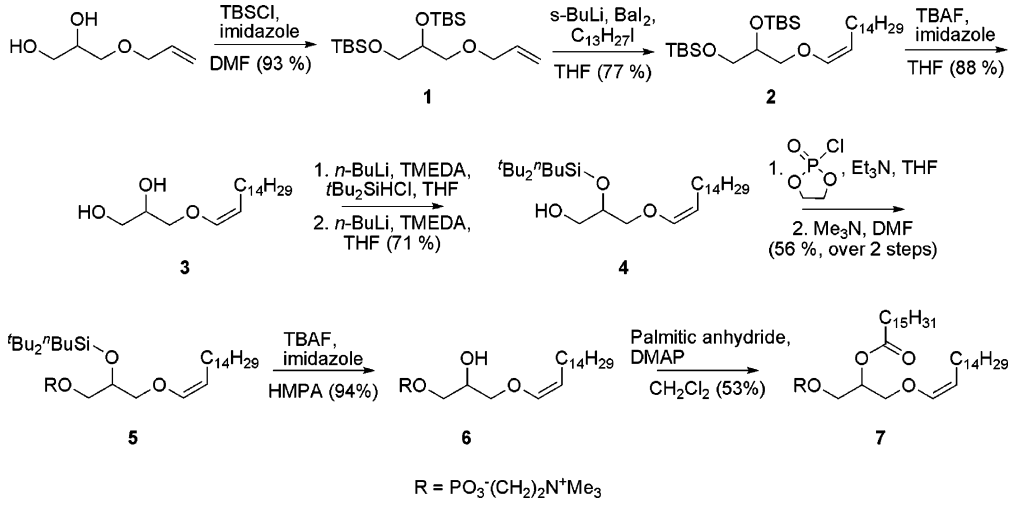
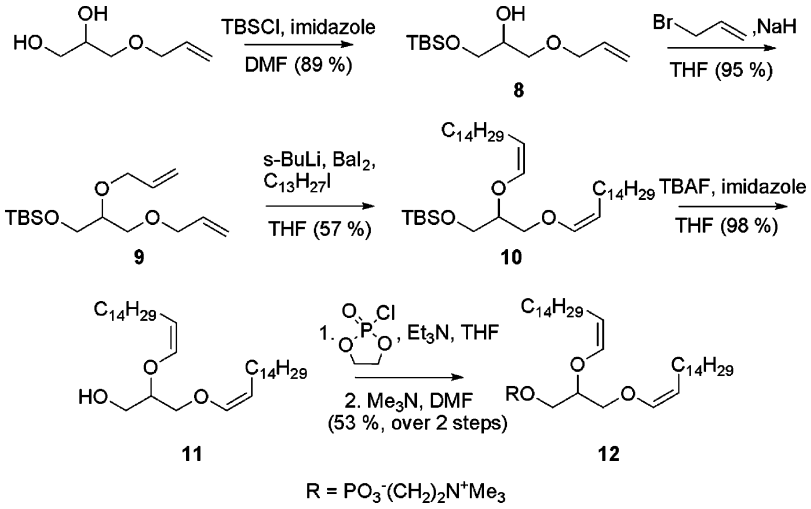
SCHEME 1. Synthesis of Plasmenylcholine



SCHEME 2. Synthesis of Bisplasmenylcholine



reagents.[20] This prompted us to modify our synthetic pathway to employ Li−Ba transmetalation in an attempt to improve the overall yield of the plasmalogen synthesis. We selected doubly TBS-protected allyl glycerol **1** as our precursor. Silyl protective groups are of particular interest in the synthesis of plasmalogens because deprotection conditions do not lead to decomposition of the vinyl ether. Deprotonation of **1** with *s*-BuLi at −78 °C produces a yellow-orange solution of the lithium allyl intermediate. The corresponding organobarium reagent is then formed by dropwise addition of an anhydrous BaI_2/THF solution at −78 °C, resulting in an orange-brown suspension. Slow addition of tridecyl iodide gave the desired γ-alkylated product **2** in high yield, with no detectable α-alkylation product observable by ^{1}H NMR.[21] This methodology was also successfully adapted to the double alkylation of bisallyl ether precursors. Prior attempts to form lithioalkoxyallyl reagents resulted in very poor yields (14%) of the desired bisplasmenyl intermediate. BaI_2 transmetalation significantly improves this transformation (57% yield), thereby enabling a rapid, practical, and stereocontrolled synthesis of bisplasmenylcholines.

Many strategies for plasmenylcholine synthesis employ protecting groups at the *sn*-3 position while the *sn*-2 position undergoes acylation; the subsequent deprotection and headgroup installation steps then generate the plasmalogen of interest.[14,15] The main problem with this approach lies in the possibility of *sn*-2 acyl migration to the *sn*-3 position during deprotection or the subsequent purification and headgroup coupling steps.[14] It has been shown that one can effectively block this migration process by protecting the *sn*-2 position prior to *sn*-3 headgroup modification.[16,17] After *sn*-2 deprotection, the product lysolipid is readily acylated with fatty acid anhydrides to produce different plasmalogen derivatives. Orthogonal protection using SEM and TBS proctection groups was previously employed in the synthesis of plasmenylcholine.[17] We sought an alternative route to effect a similar *sn*-2 protection because of the expense of SEMCl. The inside silylation method of Tanino et al.[24] allows for the protection of

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(21) Reproduction of this reaction proved difficult at first. We believe this is because of the source of BaI_2 (either isomerization or alkylation was observed). Anhydrous BaI_2[23] is freshly prepared by drying of BaI_2 hydrate in vacuo at high temperatures.[14] We found that heating (>170 °C) under high vacuum for 2 days was required for reproducible results, as was suggested in ref 22.[22]

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a vicinal diol as a five-membered cyclic silyl ether by treatment with *t*-Bu_2SiHCl and *n*-BuLi. Selective deprotection of the *sn*-3 site occurs by reaction with a second equivalent of *n*-BuLi. This method avoids the use of additional protecting groups and produces a *sn*-2 (t-Bu)$_2$-Bu silyl ether that is easily removed after *sn*-3 headgroup installation by overnight treatment with TBAF.

Our results (Scheme 1) show that removal of both TBS groups proceeds in high yield to give vicinal diol **3**, which undergoes inside silylation to generate **4** in good yield and good regioselectivity, in agreement with the values reported by Tanino et al. (9:1 *sn*-2:*sn*-3).[24] Installation of the phosphocholine headgroup with 2-oxo-2-chloro-1,3,2-dioxaphospholane and Me_3N and TBAF deprotection leads to lysoplasmalogen **6**. Acylation of **6** with palmitic anhydride produces plasmenylcholine **7** bearing 1-*Z*-hexadecenyl and hexadecanoyl chains at the *sn*-1 and *sn*-2 positions, respectively.

The synthesis of bisplasmalogens (Scheme 2) also starts from allylglycerol, which yields **8** after selective protection of the primary alcohol and allylation of the secondary alcohol. Following BaI_2-mediated bisalkylation, the TBS group was deprotected with TBAF to give the key bisplasmenyl precursor **11,** 1,2-di(1′-2-hexadecenyl)glycerol. This intermediate was converted to the phosphocholine derivative as described above to give **12** in 19% overall yield.

In conclusion, we have described a facile pathway that enables the synthesis of both plasmenyl- and bisplasmenylcholines. The use of Li−Ba exchange prior to alkyl iodide addition significantly increases the yield of the lithioalkoxyallyl alkylation step. This method is easily adapted to produce a wide variety of systems for environmentally responsive drug and gene delivery. Work on such systems is currently in progress.

Experimental Section

2,3-Di-*tert*-butyldimethylsilyl-1-*O*-1′-(*Z*)-hexadecenyl-*rac*-glycerol (2). *s*-BuLi (4.6 mL, 6.4 mmol) was added dropwise to a stirred solution of **1** (2.2 g, 6 mmol) in 100 mL of THF at −78 °C. After 30 min, a 85 mM solution of BaI_2 in THF (75 mL, 6.4 mmol) was slowly added, and the mixture was stirred for an additional 45 min at - 78 °C.[21] A solution of pentadecyl iodide (2.0 g, 6.4 mmol) in 200 mL of Et_2O was added in a dropwise manner and stirred for an additional 30 min at −78 °C. After it was stirred for an additional 30 min at −40 °C and 10 min at 0 °C, the reaction was quenched by the addition of 100 mL of hexane and 50 mL of water at 0 °C. The water phase was extracted with hexanes (3 × 100 mL), and the combined organic phases were collected, dried over K_2CO_3, and evaporated. This yielded a crude mixture, which was purified using silica gel chromatography with a step gradient of 9:1 hexanes:CH_2Cl_2 to 4:1 hexanes:CH_2Cl_2. Yield: 2.6 g; 77%; R_f = 0.3; clear oil; ^{1}H NMR ($CDCl_3$): 6.00 (d, J = 6 Hz, 1H), 4.35 (quart, J = 6 Hz, 1H), 3.93−3.84 (m, 2H), 3.74−3.60 (m, 3 H), 2.12 (quart, J = 6 Hz, 2H), 1.32 (m, 24H), 0.97−0.93 (m, 21H), 0.15−0.11 (m, 12H); ^{13}C NMR ($CDCl_3$): 145.8, 106.6, 74.5, 72.8, 64.95, 32.2, 30.2, 30.0, 29.7, 26.2, 26.1, 24.35, 23.0, 18.6, 14.4, −4.5, −5.1; ESI (M + H)$^+$ calcd 543.5, found 543; Anal. Calcd for $C_{31}H_{66}O_3Si_2$: C, 68.57; H, 12.25; Si, 10.34; Found: C, 68.82; H, 12.30; Si, 10.05.

1-*O*-1′-(*Z*)-Hexadecenyl-*rac*-glycerol (3). TBAF (13 mL, 13 mmol) was added to a solution of **2** (1.8 g, 3.3 mmol) and imidazole (1.3 g, 19 mmol) in 100 mL of THF. After stirring at room temperature for 3 h, 75 mL of water and diethyl ether were added. After separation, the aqueous phase was washed three times with 100 mL of EtOAc. The combined organic phases were dried over K_2CO_3 and evaporated. The crude product was purified with silica gel chromatography using EtOAc as eluent. Yield: 910 mg, 88%; R_f = 0.5; white solid; ^{1}H NMR ($CDCl_3$): 6.01 (dt, J = 6, 1 Hz, 1H), 4.48 (quart, J = 6 Hz, 2H), 4.0 (m, 1H), 3.88−3.73 (m, 4H), 2.6 (s, 1 H), 2.12 (quart, J = 6 Hz, 2H), 1.32 (s, 24H), 0.95 (t, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$): 144.7, 108.6, 73.45, 70.8, 63.85, 32.2, 29.9, 29.8, 29.6, 24.2; 22.95, 21.5; 14.4; ESI (M + Na)$^+$ calcd 337, found 337.

1-*O*-1′-(*Z*)-Hexadecenyl-2-(butyl-di-*tert*-butylsilyl)-*rac*-glycerol (4). *n*-BuLi (0.75 mL, 1.9 mmol), TMEDA (0.49 mL, 3.3 mmol), and tBu_2SiHCl (0.384 mL, 1.9 mmol) were successively added to an ice-cooled flask containing **3** (400 mg, 1.3 mmol) in 50 mL of THF. After stirring for 7 h at reflux, the mixture was cooled to −78 °C and *n*-BuLi (1.5 mL, 3.8 mmol) was added. The solution was stirred for an additional 2 h at −78 °C and 1 h at −45 °C. After reaction, water (50 mL) and ether (50 mL) were added and the mixture was separated. The aqueous phase was extracted with ether, and the combined organic phases were dried over K_2CO_3. Filtration and evaporation yielded a crude mixture that was purified with column chromatography using 20% diethyl ether in hexane as eluent. Yield: 469 mg; 71%; R_F=0.4 (the *sn*-3 regioisomer can be isolated with R_f = 0.43); clear oil; ^{1}H NMR ($CDCl_3$): 5.98 (dt, J = 6, 1 Hz, 1H), 4.42 (quart, J = 6 Hz, 2H), 4.10 (m, 1H), 3.87−3.75 (m, 4H), 2.11 (quart, J = 6 Hz, 2H), 2.03 (dd, J = 6, 3 Hz, 1H), 1.67−1.50 (m,2 H), 1.45−1.33 (m, 26H), 1.14−1.09 (m, 18 H), 1.03−0.93 (m, 6H), 0.9−0.85 (m, 2H); ^{13}C NMR($CDCl_3$): 145.0, 107.8, 72.9, 71.5, 64.2, 32.2, 30.0, 29.8, 29.63; 28.6, 27.7, 27.2, 24.3, 23.0, 21.5, 14.4, 13.9, 11.52; ESI (M + H)$^+$ calcd 535.5, found 535; Anal. Calcd for $C_{31}H_{64}O_3Si$: C, 72.59; H, 12.58; Si, 5.48; Found: C, 72.79; H, 12.58; Si, 5.31.

1-*O*-1′-(*Z*)-Hexadecenyl-2-(butyl-di-*tert*-butylsilyl)-*rac*-glycero-3-phosphocholine (5). Triethylamine (1 mL, 13 mmol) and 2-oxo-2-chloro-1,3,2-dioxaphospholane (0.286 mL, 3.1 mmol) were added to a flask containing **4** (400 mg, 0.78 mmol) in THF at 0 °C. The solvent was removed under vacuum after stirring at 25 °C for 30 min. The residue was transferred to a pressure vessel with 15 mL of DMF. Trimethylamine (∼10 mL) was condensed into the vessel with liquid nitrogen cooling, and the mixture was stirred at 70 °C for 24 h. After slow release of high pressure at 0 °C, the resulting solution was evaporated and the residue was purified using a silica gel column (63:32:5 CH_2Cl_2:MeOH:H_2O). Suspended silica gel from the chromatographic fractions was removed using a 0.45 μm PTFE syringe filter to give a white solid after lyophilization from benzene. Yield: 362 mg, 68%; R_f = 0.3; white solid, ^{1}H NMR ($CDCl_3$): 6.0 (d, J = 6, 1H), 4.34 (m, 3H); 4.15 (m, 1H), 3.97−3.81 (m, 6H), 3.42 (s, 9H), 3.2 (m, 1H), 2.10 (m, 2H), 1.57−1.50 (m, 2H), 1.40−1.33 (m, 26H), 1.07 (m, 18 H), 0.99−0.92 (m, 6H), 0.88−0.82 (m, 2H); ^{13}C NMR ($CDCl_3$): 145.4, 106.5, 74.2, 59.2, 54.5, 32.0, 29.8, 29.5, 28.5, 28.4, 27.5, 27.0, 24.3, 22.7, 21.4, 14.2, 13.8, 11.3, −8.523; ^{31}P ($CDCl_3$): −62.4; ESI (M + H)$^+$ calcd 678.5, found 678. Anal. Calcd for $C_{31}H_{76}NO_6Si.(H_2O)_2$: C, 60.55; H, 11.29; N, 1.96; P, 4.34; Si, 3.93; Found: C, 60.89; H, 11.53; N, 1.99; P, 4.30; Si, 3.81.

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Supporting Information Available: Detailed descriptions of the synthetic methods for compounds **1**, **6**−**12** and spectra for all isolated compounds are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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