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SYNTHESIS AND PROPERTIES OF A PLASMALOGEN QUINONE

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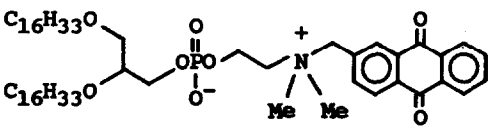
Abstract: A novel phospholipid quinone bearing hexadecyl ether chains has been synthesized and employed in the preparation of redox-active, quinone-functionalized liposomes.

Respiratory and photosynthetic energy transduction[1] involve the transport of electron across a phospholipid bilayer membrane by membrane-bound quinones. Adopting the approach of using functionalized liposomes[2] as realistic models of biomembrane assemblies, we have initiated the preparation and characterization of quinone-functionalized liposomes[3]. We report herein the multi-step chemical synthesis of a phospholipid quinone (1) containing hexadecyl ether linkages (i.e., plasmalogen). (1) assembles with simple phospholipids like dipalmitoyl-phosphatidylcholine (DPPC) or dioleoylphosphatidylcholine (DOPC) to form quinone-functionalized liposomes that are reduced and reoxidized by solution reactants. The route employed here is more versatile than the chemical modification method described previously[3] and provides us with a means of preparing phospholipid quinones with varying and controllable structures. Functionalized liposomes prepared from such specifically-designed phospholipids should provide simple, chemical models for the redox and transport reactions of membrane-bound quinones within respiratory and photosynthetic electron transport systems.

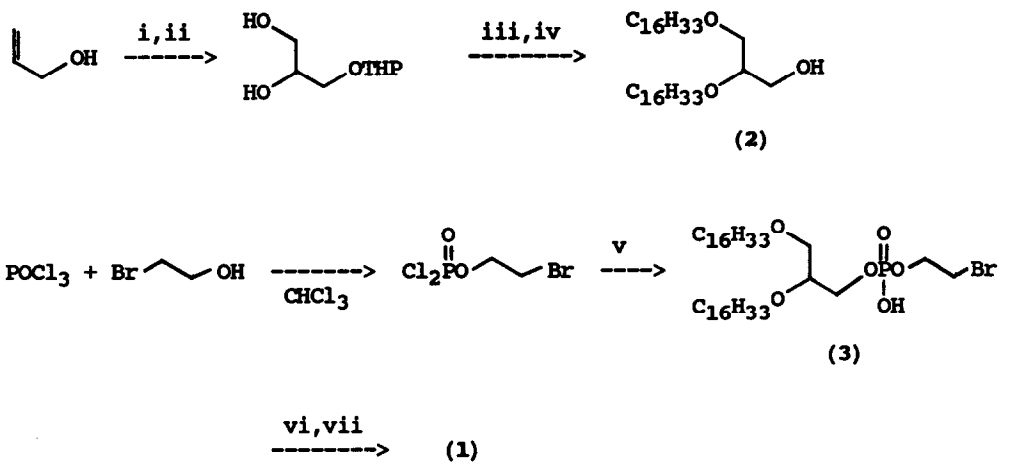


(1)

The synthetic route to (1) is shown in SCHEME I. Allyl alcohol is protected[4] with 3,4-dihydropyran (DHP) and oxidized[4] with $KMnO_4$ to yield 1-(2-tetrahydro-pyranyl)-glycerol. This protected glycerol is alkylated with NaH/DMF and $C_{16}H_{33}I$, then deprotected[5] to yield the 1,2-dihexadecylglycerol (2). The excess $C_{16}H_{33}I$ is removed from (2) by chromatography on SiO_2 with hexanes, then 3:1 $CCl_4/CHCl_3$, to yield white, waxy flakes, containing less than 3% $C_{16}H_{33}I$ impurity (^1H NMR). Reaction of (2) with 2-bromoethyldichlorophosphate[6] yields the bromoethyl phospholipid intermediate (3). (3) is reacted with Me_2NH in acetone[7] and 2-

bromomethylanthraquinone[8] ($BrCH_2AQ$) in 1:1 $MeOH/CHCl_3$ to complete the synthesis. (1) is purified by chromatography on SiO_2 with $CHCl_3/MeOH/H_2O$ (79:20:1, then 74:24:2), extraction with 0.002 M H_2SO_4 and aqueous EDTA, and lyophilization from C_6H_6. Final yields of the amorphous, white product are typically 45-65 %. The 300 MHz ^1H NMR spectrum in $CDCl_3$ reveals the aromatic resonances between 7.6 and 8.3 ppm (1:2:2:2 ratio), the glycerol, choline, and α-CH_2 resonances between 3.2 and 5.1 ppm (1:2:2:2:8:6 ratio), and the alkyl chain resonances (β - ω) at 1.3 ppm (4H), 1.1 ppm (52H), and 0.75 ppm (6H). Significant amounts of water (4 - 10 H_2O / (1)) are also observed. CHN microanalysis verifies the proposed formula and the presence of H_2O.

SCHEME I



(i) DHP, cat. HCl. (ii) $KMnO_4/H_2O$. (iii) NaH/DMF; $C_{16}H_{33}I$. (iv) cat. $HCl/Et_2O/MeOH$. (v) (2)/Et_2O. (vi) Me_2NH/acetone, 4 h, 70°C. (vii) $BrCH_2AQ/CHCl_3/MeOH$, 18 h, 60°C.

Sonication[3] of phopspholipid mixtures containing 1-15 mol% (1) and DPPC (at 52°C) or DOPC (at room temperature) in N_2-blanketed 0.2 M KCl with 0.050 M Tricine (pH 8) and fractionation of the clarified suspensions on Sephadex G50 yield unilamellar, quinone-functionalized liposomes. DPPC liposomes are maintained at 52°C to maintain the liquid crystalline phase (T_c = 42°C[9a]), while DOPC liposomes can be used at room temperature. These quinone-functionalized liposomes possess optical and size exclusion chromatographic characteristics identical with DPPC[9a] or DOPC liposomes, so the functionalized liposomes should possess an average diameter of ca. 25 nm[9b]. The liposome-bound quinones can be reduced and reoxidized rapidly and reversibly by chemical agents. Figure 1 illustrates the UV-Vis spectra following sequential additions of aqueous $S_2O_4^{2-}$ to a 10 mol%

(1)/DOPC liposome solution. The quinone peak (λ_{max} = 324 nm) decreases, while the hydroquinone peak (λ_{max} = 388 nm) increases, upon addition of reductant until all of the quinone is reduced (curve 3). Exposure of the solution to oxygen or addition of $Fe(CN)_6^{3-}$ (curve 4) regenerates the quinone peak. The quinone reduction and reoxidation reactions proceed with half-lives of ca. 5 sec at room temperature. BH_4^- also reduces the quinones; however, unlike $S_2O_4^{2-}$, BH_4^- does not penetrate the bilayer surface[9a]. Since only 90 % of the quinones are reduced with BH_4^-, it is natural to conclude that 90 % of the quinones reside on the outer surface of the liposome.

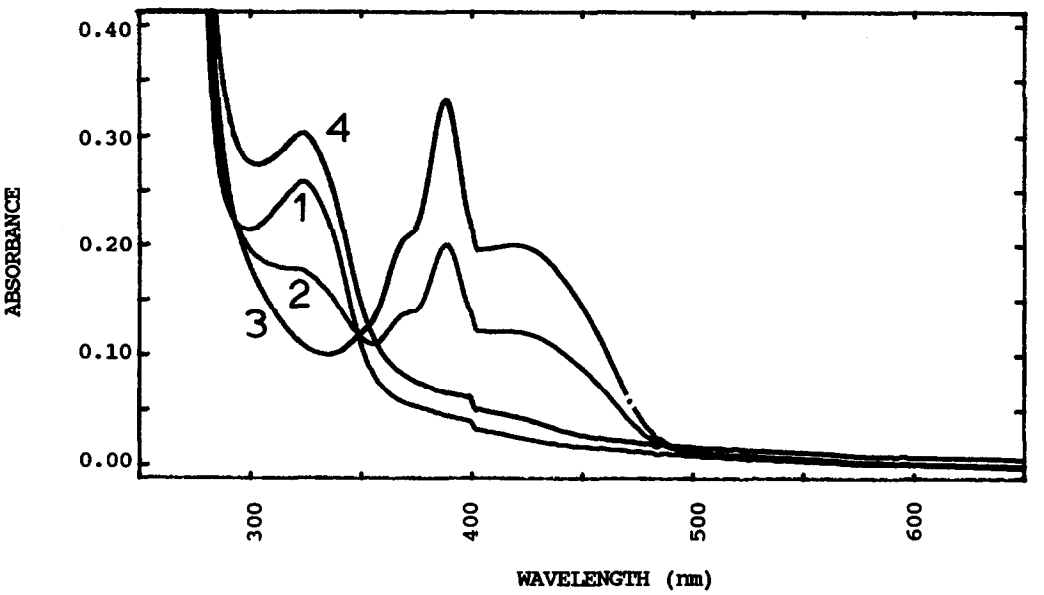


Figure 1. Curve 1: initial; curve 2: 0.5 equiv. $S_2O_4^{2-}$; curve 3: 1.0 equiv $S_2O_4^{2-}$; curve 4: ca. 1.05 equiv. $Fe(CN)_6^{3-}$.

Further characterization of the structural, redox, and transport properties of these new biomimetic assemblies will come from additional spectrophotometric kinetics, variable-temperature NMR, and thermal methods. We are presently extending this versatile, synthetic method to prepare analogous phospholipid quinones with varying chain lengths and quinone position. Quinone functionalized liposomes derived from such phospholipid quinones should model the location, position, and reactivity of ubiquinone and plastiquinone with respiratory and photosynthetic energy transduction systems.

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