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Progress toward the synthesis of a biomimetic membrane

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Abstract

Ubiquinone has antioxidant properties and is important in the conversion of products from glycolysis and the citric acid cycle to ATP. We report the synthesis of the necessary components of a biological membrane mimic that can serve as a model system for elucidating the third step in the prokaryotic biosynthesis of ubiquinone.

The electron-transport chain converts the products of glycolysis and the citric acid cycle into energy in the form of ATP. A key component in this process is ubiquinone (coenzyme Q or Q).[†] Existing as a nonpolar molecule that diffuses freely within the extensive mitochondrial framework, ubiquinone is an essential lipid component of the mitochondrial electron-transport chain of eukaryotes and the plasma membrane of many prokaryotes.[1] Q is also present in other intracellular membranes and human low-density lipoproteins.[2] It has been shown that the reduced form of Q (QH_2), the hydroquinone, functions as an antioxidant by scavenging lipid peroxy radicals, a class of oxidative products that represents a major cause of damage to cellular membranes. Oxidative damage to mammalian cells has been linked to cancer and age-related degenerative diseases.[3] Despite the importance of Q/QH_2, many aspects of Q biosynthesis are still incomplete.

Our approach to investigate the biosynthesis of ubiquinone, specifically the step catalyzed by an *O*-methyltransferase[4] in the presence of *S*-adenosylmethionine, is to use self-assembled monolayers (SAMs) to model the inner-mitochondrial membrane (Fig. 1). We report here the synthesis of the key components of this biological membrane: an alkanethiol phosphonic acid, which mimics the phosopholipid component,[5] and an alkenethiol ubiquinone precursor tether. The mixed monolayer assembly on gold provides an excellent scaffold to probe the prokaryotic enzyme-binding site in the third step of ubiquinone biosynthesis.

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† Abbreviations: Q, ubiquinone; QH2, ubiquinol.

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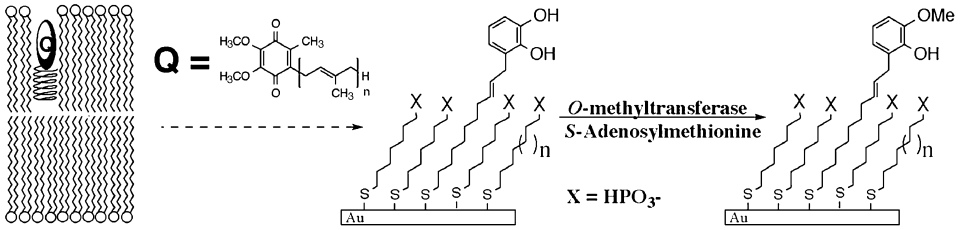
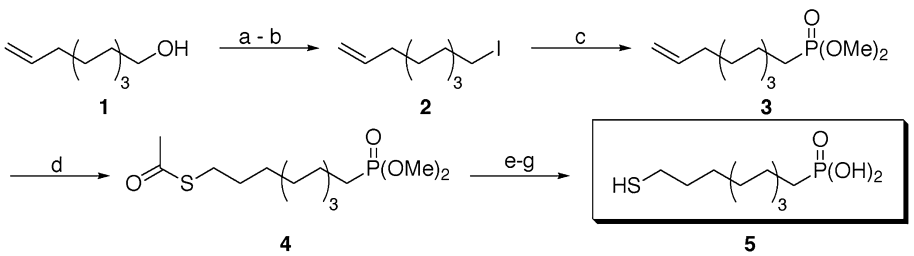


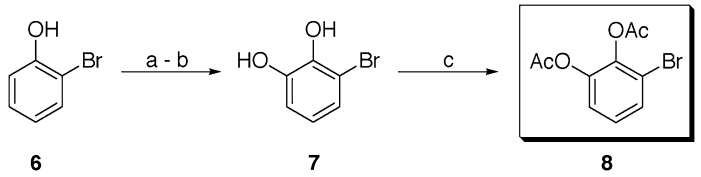
Figure 1. SAM of ubiquinone precursor

The synthesis of the alkanethiol phosphonic acid is initiated by the addition of *p*-toluenesulfonyl chloride to the alcohol of **1** followed by displacement with sodium iodide (NaI) to furnish 10-iododecene **2** (Scheme 1). Refluxing the iodo species in the presence of excess trimethyl phosphite, a modified Michaelis–Arbuzov reaction, gives the dimethyl phosphonate **3**.[6] The thiol linkage is afforded by hydrothioacetylation of alkene **3**, thiolacetic acid and 2,2′-azobisisobutyronitrile (AIBN),[7] to give **4**.[8] Methanolysis of the isolated thiolacetate followed by the facile dealkylation of the resulting thiol dimethylphosphonate using bromotrimethylsilane yields **5**, 10-thioldecane-phosphonic acid, in 95% yield.[9]



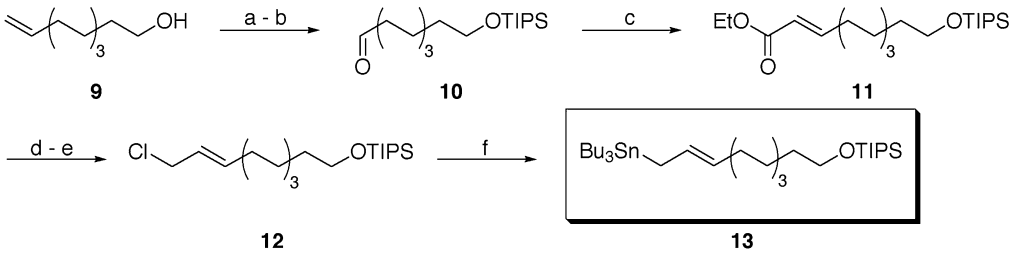
Scheme 1. (a) TsCl, Et_3N, DMAP, CH_2Cl_2, 96%; (b) NaI, acetone, 96%; (c) $P(OMe)_3$ (10 equiv.), reflux, 95%; (d) thiolacetic acid, AIBN, Tol, reflux, 87%; (e) acetyl chloride, MeOH, 0°C, 85%; (f) TMS-Br; (g) H_2O, 95% (two steps).

Synthesis of the ubiquinone analog tether begins with commercially available *o*-bromophenol **6** (Scheme 2). Using chloroform ($CHCl_3$), then sodium hydroxide (NaOH), the *o*-aldehyde is formed via the Reimer–Tiemann reaction.[10] Dakin oxidation of this aldehyde with hydrogen peroxide (H_2O_2) and NaOH affords 3-bromocatechol **7**.[11] Protection of the free hydroxyl groups with acetic anhydride (Ac_2O) yields the desired 1,2-diacetoxy-3-bromobenzene **8**.[12]



Scheme 2. (a) $CHCl_3$, NaOH, reflux; (b) H_2O_2, NaOH, 12% (two steps); (c) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 85%

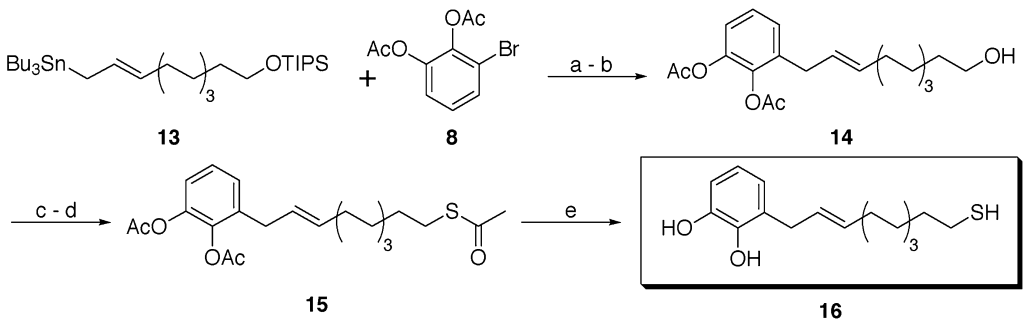
The construction of the allylic stannane **13** also begins with commercially-available 9-decen-1-ol **1** (Scheme 3). Protection of this alcohol with triisopropylsilyl triflate (TIPS-OTf) followed by ozonolysis of the alkene gives **10**. The aldehyde is homologated using triethyl phophonoacetate to yield the allylic ester **11**.[13] Diisobutylaluminum hydride reduction (DIBAL-H) followed by halogenation with *N*-chlorosuccinimide (NCS) and dimethyl sulfide (DMS) of the isolated alcohol produces the allylic chloride **12**.[14] Other carbon-activating methods, such as Ph_3P/Br_2, Ph_3P/CBr_4, and MsCl, were found to remove the -TIPS protecting group or, in the case of the latter, serve in the promotion of an undesired S_N2' reaction.[15] The anion of tributyltin hydride is added to **12** to afford the allylic stannane **13**.[13]



Scheme 3. (a) TIPSOTf, Et_3N, 0°C→rt, CH_2Cl_2, 99%; (b) O_3, CH_2Cl_2, –78°C, then nBu$_3$P, 96%; (c) nBuLi, triethyl phosphonoacetate, Et_2O, 0°C, 97%; (d) DIBAL-H, CH_2Cl_2, –78°C, 91%; (e) NCS, DMS, CH_2Cl_2, –20°C, 75%; (f) LDA, Bu_3SnH, THF, 0°C, 80%

Arylbromide **8** and allylic stannane **13** are joined via a Stille coupling (Scheme 4).[16] This reaction affords the catechol derivative **14** after the selective deprotection of the triisopropylsilyl group with tetrabutylammonium fluoride (TBAF). Upon activation of this alcohol with methanesulfonyl chloride (MsCl), the protected thiol **15** is afforded by the introduction of potassium thiolacetate. The simultaneous deprotection of all acetyl groups by treatment with potassium carbonate (K_2CO_3) in methanol[17] yields the desired thiolated-alkenyl ubiquinone precursor **16**.[18]

In summary, we have developed the components necessary to create a biological membrane mimic. We are currently employing ubiquinone precursors of various lengths, synthesized by the strategies described within, to probe the binding site of enzymes used in the biosynthesis of Q.



Scheme 4. (a) $Pd(PPh_3)_4$, HMPA, 65°C, 78%; (b) TBAF, THF, 85%; (c) MsCl, TEA, CH_2Cl_2, 0°C→rt; (d) potassium thiolacetate, THF, 92% (two steps); (e) K_2CO_3, MeOH, 95%

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18. Satisfactory spectroscopic data was obtained for all intermediates. Acid **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (m, 18H), 2.52 (dt, 2H, $J=7.4$, 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7, 28.2, 28.4, 28.4, 28.7, 29.1, 29.4, 29.5, 32.8, 34.1; ^{31}P NMR (160 MHz, MeOH, dicyclohexylammonium salt) δ 24.3; HRMS m/z calcd for $C_{10}H_{23}O_3PS$ (M^+): 254.1106, found 254.1099. Thiol **16**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (m, 12H), 2.06 (q, 2H), 2.48 (dt, 2H, $J=7.4$, 7.4 Hz), 3.25 (d, 2H), 5.44 (br s, 2H), 5.61 (m, 1H), 5.76 (m, 1H), 6.72 (m, 3H); HRMS m/z calcd for $C_{17}H_{26}O_2S$ (M^+): 294.1637, found 294.1637.