Design, Synthesis, and Photochemical Properties of a Photoreleasable Ubiquinol-2: A Novel Compound for Studying Rapid Electron-Transfer Kinetics in Ubiquinol-Oxidizing Enzymes

Michael H. B. Stowell,*,†,‡ Guangyang Wang,† Michael W. Day,§ and Sunney I. Chan*,†

Contribution from the Arthur Amos Noyes Laboratory of Chemical Physics, the Beckman Institute, and the Carl F. and Winifred H. Braun Laboratories, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received April 4, 1997

Abstract: The design and multistep convergent synthesis of the novel photoactive ubiquinol-benzoin adduct **1a,b** has been accomplished. Optical spectra of the steady-state photolysis reactions showed a smooth conversion from **1a,b** to 5,7-dimethoxy-2-phenylbenzofuran (**13**) and ubiquinol-2 with an isobestic point at 258 nm. HPLC analysis of the photoproducts was also consistent with the clean formation of the desired ubiquinol-2 (**3**) and the expected 5,7-dimethoxy-2-phenylbenzofuran (**2**). Transient photolysis at 355 nm was consistent with a rapid photolysis rate that exceeded the instrument response time ($>10^6$ s^{-1}). Accordingly, the study of rapid electron-transfer events in ubiquinol oxidizing enzymes is now feasible. Furthermore, the synthetic methods developed herein will be of general application for the facile synthesis of a variety of photoreleasable substrates for studying rapid kinetic events in enzymatic reactions.

Introduction

The study of rapid electron-transfer events in redox active enzymes is critical to understanding the detailed enzymatic mechanism of these proteins. An example is cytochrome *c* oxidase where the methods for rapid photoreduction of cytochrome *c* have facilitated the investigation of the electron input events as well as intramolecular electron-transfer processes in this enzyme.[1−7] While such methods are well suited for single electron donating or accepting proteins such as cytochrome *c*, a number of redox active enzymes are ubiquinol oxidizing enzymes and are not amenable to these methods because they require a rapid two electron reduction of ubiquinone to form ubiquinol in order to initiate the chemistry. A novel approach to this problem was the use of the photosynthetic reaction center (PRC) to produce ubiquinol from ubiquinone and to study the rapid kinetic events in the b_6f complex[8]. Unfortunately, the rate for ubiquinol release from the PRC is slow in comparison to typical ubiquinol oxidation rates for enzymes such as the bc_1 or b_6f complex. Consequently, a more rapid method is required for producing ubiquinol on a sufficiently fast time scale to make kinetic resolution possible.

Toward this end, we have developed a method that can rapidly produce ubiquinol through the use of "caged" compounds. Caged compounds were originally utilized as protecting groups in organic synthesis and have the convenient property that they can be removed photochemically. In a classic paper, Woodward described the synthesis and photocleavage properties of a series of substituted nitrobenzyl protecting groups.[9] Subsequently, a variety of nitrobenzyl protecting groups have played important roles in synthetic strategies as well as being exploited for use in such diverse fields as semiconductor lithography[10] and as "caged" compounds in the study of rapid enzymatic processes.[11−13]

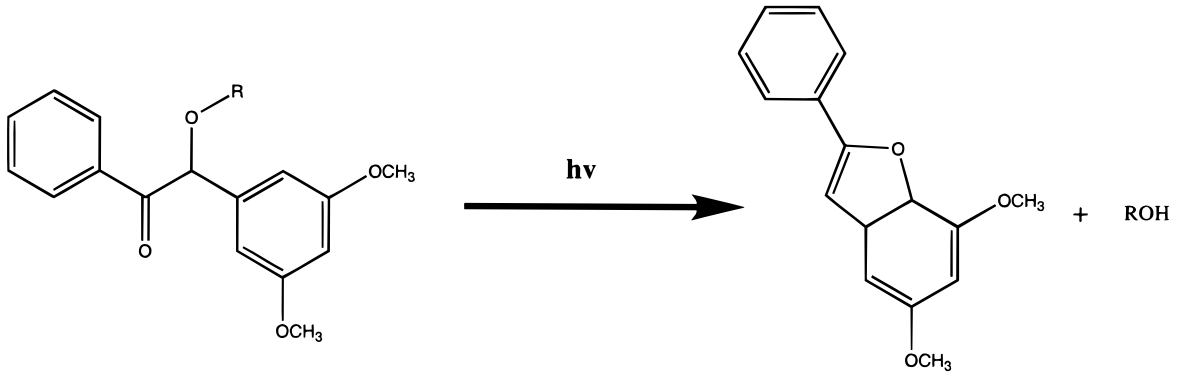
Recently, a recurring interest in 3′,5′-dimethoxy benzoin (DMB) compounds has been generated as these compounds possess remarkable photolysis properties. Such compounds have been reported[14] to photolyze with rates exceeding 10^{10} s^{-1} and quantum yields of 0.67 to give 5,7-dimethoxy-2-phenylbenzofuran (**13**) and the caged substrate **ROH**, Scheme 1. Herein we report the design, synthesis, and photochemical properties of a "caged" ubiquinol-2 (**1a,b**), based upon the photoactive 3′,5′-dimethoxy benzoin. We also report the remarkable photocleavage properties of this "caged" ubiquinol and demonstrate the production of ubiquinol-2 with rates exceeding 10^6 s^{-1}. Accordingly, with this newly available caged ubiquinol-2, rapid electron-transfer studies on ubiquinol oxidiz-

* To whom correspondence should be addressed.
† Arthur Amos Noyes Laboratory of Chemical Physics.
‡ Carl F. and Winifred H. Braun Laboratories.
§ Beckman Institute.

(1) Larsen, R. W.; Winkler, J. R.; Chan, S. I. *J. Phys. Chem.* **1992**, *89*, 8023.
(2) Hazzard, J. T.; Rong, S.-Y.; Tollin, G. *Biochemistry* **1991**, *30*, 213.
(3) Tollin, G.; Hurley, J. K.; Hazzard, J. T.; Meyer, T. E. *Biophys. Chem.* **1993**, *48*, 259.
(4) Brunshwig, B. S.; Delaive, P. J.; English, A. M.; Goldberg, M.; Mayo, S. L.; Sutin, N.; Gray, H. B. *Inorg. Chem.* **1985**, *24*, 3743.
(5) Orii, Y. *Biochemistry* **1993**, *32*, 11910.
(6) Pan, L.-P.; Hazzard, J. T.; Lin, J.; Tollin, G.; Chan, S. I. *J. Am. Chem. Soc.* **1991**, *113*, 5908.
(7) Pan, L. P.; Hibdon, S.; Liu, R.-Q.; Durham, B.; Millet, F. *Biochemistry* **1993**, *32*, 8492.
(8) Prince, R. C.; Matsuura, K.; Hurt, E.; Hauska, G.; Dutton, P. L. *J. Biol. Chem.* **1982**, *257*, 3379.
(9) Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333.
(10) Sabomgi, G. J. *Chemical Triggering*; Plenum: New York, 1987.
(11) Gurney, A. M.; Lester, H. A. *Physiol. Rev.* **1987**, *67*, 583.
(12) McCray, J. A.; Trentham, D. R. *Annu. Rev. Biophys. Chem.* **1989**, *18*, 239.
(13) Kaplan, J. H., Forbusch, B. III, Hoffman, J. F. *Biochemistry* **1978**, 17, 1929.
(14) Ruttiman, A.; Lorenz, P. *Helv. Chim. Acta* **1990**, *73*, 790.

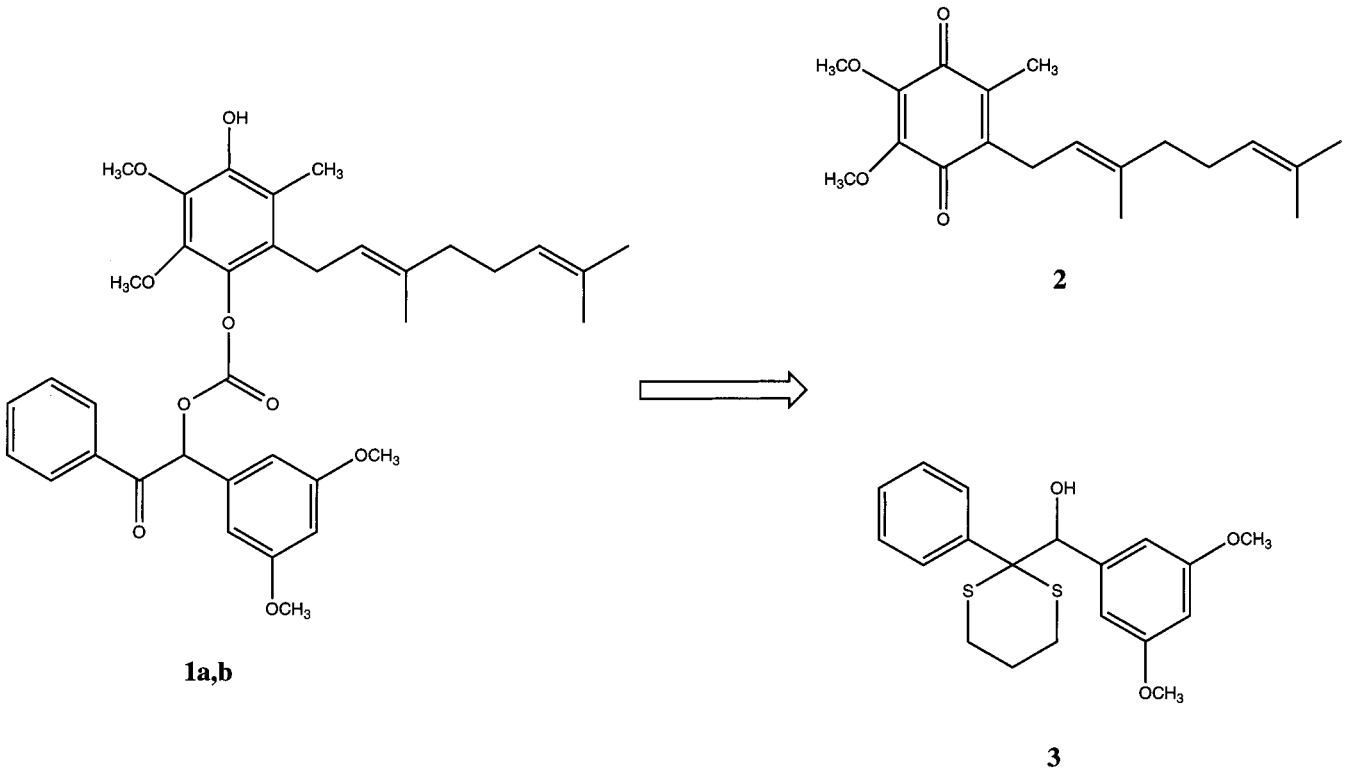
S0002-7863(97)01079-2 CCC: $15.00
Published on Web 03/04/1998

Scheme 1



1 **13**

Scheme 2



1a,b **2** **3**

ing enzymes are now feasible, which will lead to a greater understanding of this important class of enzymes.

Results and Discussion

In designing a system for rapidly producing ubiquinol by flash photolysis, several factors were considered. First, to ensure kinetic resolution, the photolysis rate must exceed the enzymatic turnover rate by several orders of magnitude. Second, the quantum yield of photolysis should be large enough to produce readily measurable signals for kinetic studies by transient absorption. Last, the photolysis should minimize secondary radicals or other reactive species that could inhibit or damage the protein sample. For these reasons, the use of the DMB compounds, originally described by Sheehan and co-workers,[15] seemed ideal. These compounds exhibit very rapid photolysis rates ($>10^{10}$ s^{-1}, for the acetates), with a high quantum efficiency of 0.64, and in addition, the benzofuran photolysis product is stable and unreactive. A simple retrosynthetic scheme leads to ubiquinone-2 (**2**) and the readily available[16] dithiane (**3**).

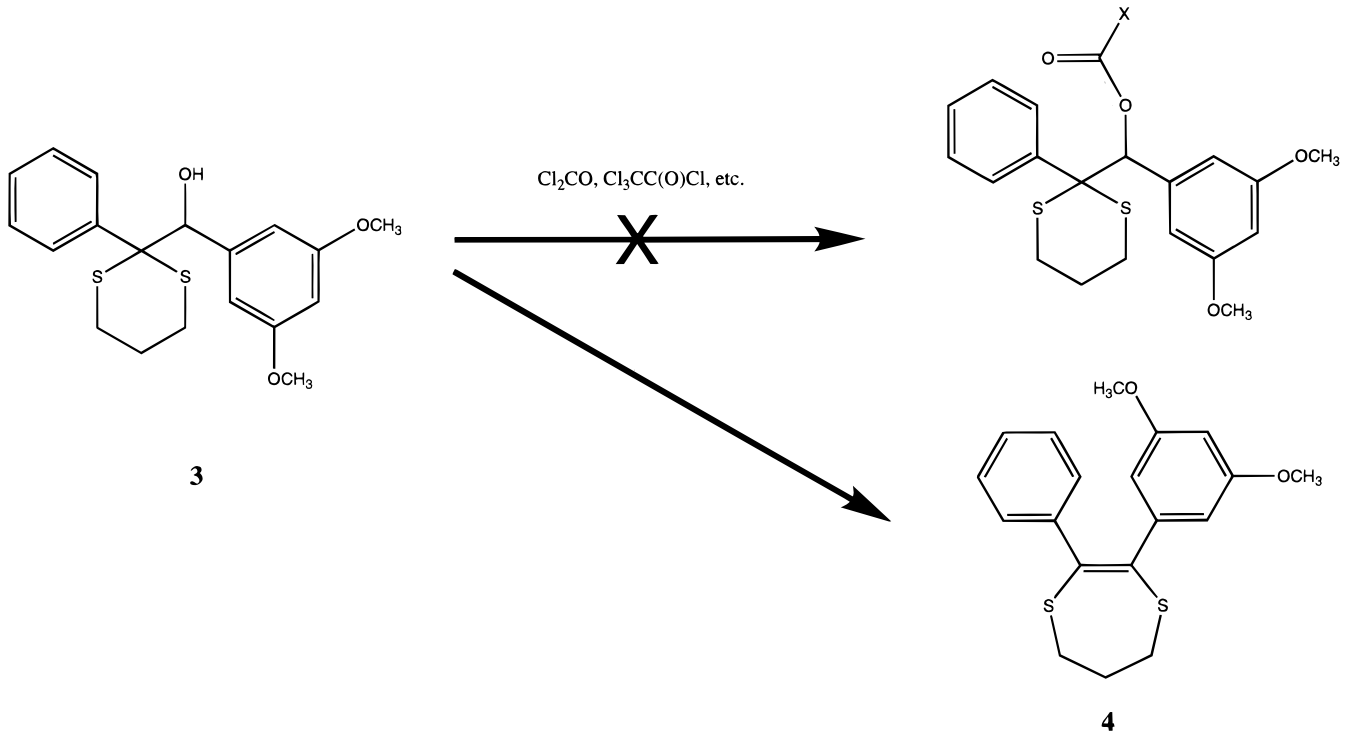
Synthesis of a DMB Coupling Reagent. The crtitical step in this scheme is the formation of the carbonate linkage between the ubiquinol and the dithiane **3**. Several methods were investigated for the formation of an activated carbonate dithiane. These included the use of phosgene, di- and tri-phosgene, and carbonyl diimidazole as well as the triflate salt of dimethyl carbonyl diimidazole. These methods were pursued but ultimately were deemed inefficient for our purposes due to the formation of a dehydration product (**4**) in substantial, to near quantitative yield.

We then attempted to form the mixed carbonate of 2-nitrophenol and dithiane **3**. The 2-nitrophenol carbonate was chosen

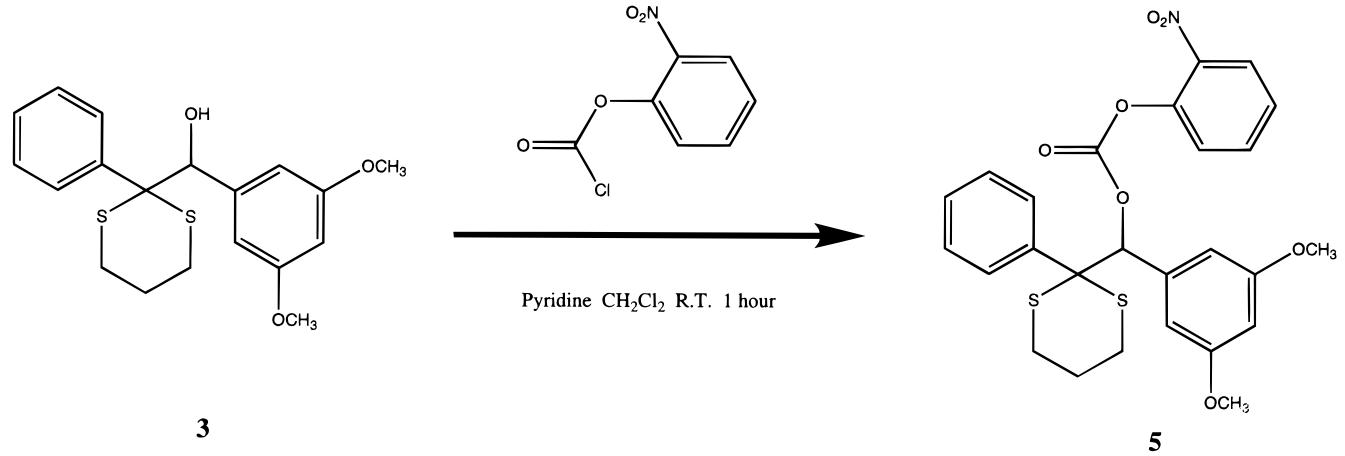
(15) Sheehan, J. C.; Wilson, R. M.; Oxford, A. W. *J. Am. Chem. Soc.* **1971**, *93*, 7222.

(16) Stowell, H. H. B.; Rock, R. S.; Rees, D. C.; Chan, S. I. *Tetrahedron Lett.* **1995,** *37*, 307.

Scheme 3



Scheme 4



because it has been reported that such carbonates are highly reactive in the presence of DMAP,[17] and we anticipated that the coupling of a hindered 2,6 substituted aromatic system would be difficult. Reaction of dithiane **3** with 2-nitrophenyl chlorocarbonate in methylene chloride and excess pyridine readily produced the desired mixed carbonate **5** in good yield and without formation of the previously observed dehydration product **4**.

With a readily available coupling compound in hand, we then attempted to establish if the coupling between compound **5** and a sterically hindered phenol would be possible. Accordingly, we reacted compound **5** with 2,6-dimethoxyphenol in the presence of DMAP at room temperature.

We were delighted to observe the nearly quantitative formation of the desired compound **6**. This compound readily crystallized and was subjected to X-ray diffraction analysis in order to firmly establish that the desired synthetic tranformations had proceeded as expected. The X-ray crystal structure of compound **6** confirms the correctness of the synthetic methods to this point. Fifty percent thermal elipsoid ORTEP drawing of the X-ray crystal structure is given in Supporting Information. It is worth noting that the ease of preparation and the observed coupling efficiency of compound **5** should make it a versatile reagent in a variety of synthetic methods where orthogonal protecting groups are required.

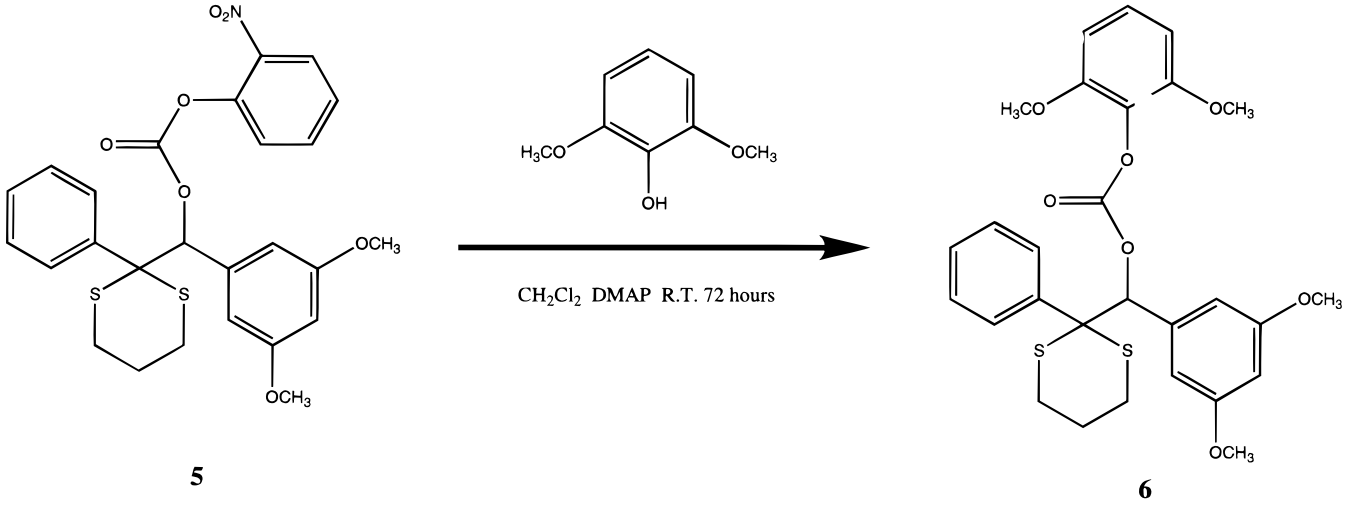
Synthesis of the Ubiquinol-2 Substrate. The next synthetic target was the ubiquinone-2 molecule. The methods utilized were similar to those described earlier[18] and utilized a Diels−Alder transformation of quinone to the tricyclodione (**7**). Alkylation and subsequent retro-Diels−Alder reaction of the alkylated tricyclodione (**8**) afforded the desired ubiquinone-2 in an overall yield of 73%.

Because of the bifunctional nature of the ubiquinol-2, we chose to selectively silyate a single phenolic oxygen using triethylsilyl chloride. Treatment of ubiquinone-2 with sodium

(17) Brunelle, D. J. *Tetrahedron Lett.* **1982**, *23*, 1739.

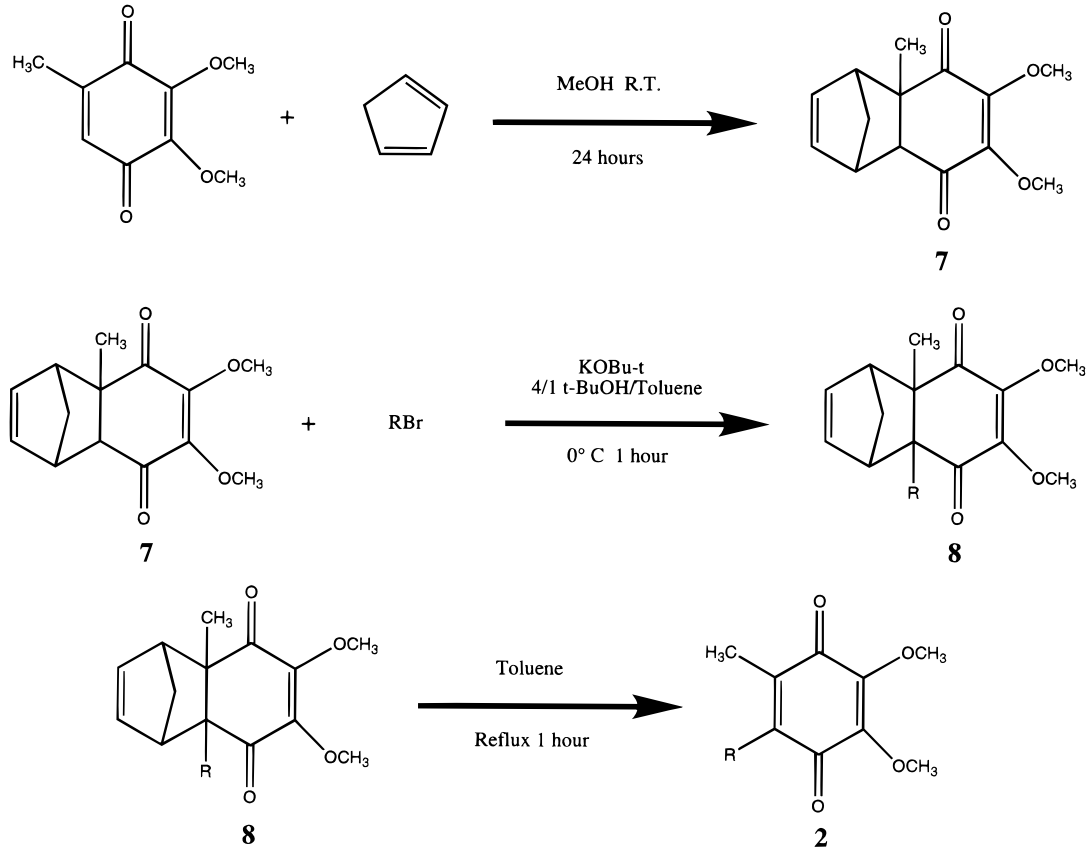
(18) van Liemt, W. B. S.; Steggerda, W. F.; Esmeijer, R.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 153.

Scheme 5



5 **6**

Scheme 6



7 **7** **8** **8** **2**

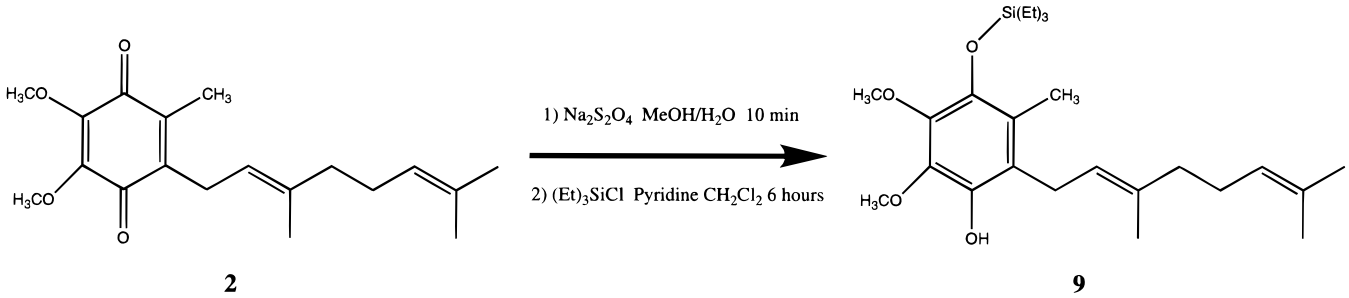
dithioinite in aqueous methanol and extraction with hexane gave ubiquinol-2 in greater than 99% yield. Silyation of the ubiquinol-2 with triethylsilyl chloride in dry acetonitrile with pyridine catalyst afforded the monosilyl ubiquinol-2 (**9**) in 61% yield.

Assembly of the Caged Ubiquinol-2. The monosilyl ubiquinol-2 was subsequently coupled to the *o*-nitrophenyl carbonate ester of 3′,5′-dimethoxyphenyl(phenyldithiane) using DMAP in methylene chloride. Formation of the *o*-nitrophenyl carbonate ester of 3′,5′-dimethoxyphenyl(phenyldithiane) was accomplished using *o*-nitrophenyl chloroformate in pyridine.

Activation and deprotection was accomplished in one step by first treatment with bisTFAiodobenzene in THF water, followed by silyldeprotection by HF:pyridine in acetonitrile. The resultant product was purified by reverse phase HPLC to yield the desired "caged" ubiquinol-2 (**1a,b**) as a clear colorless waxy solid.

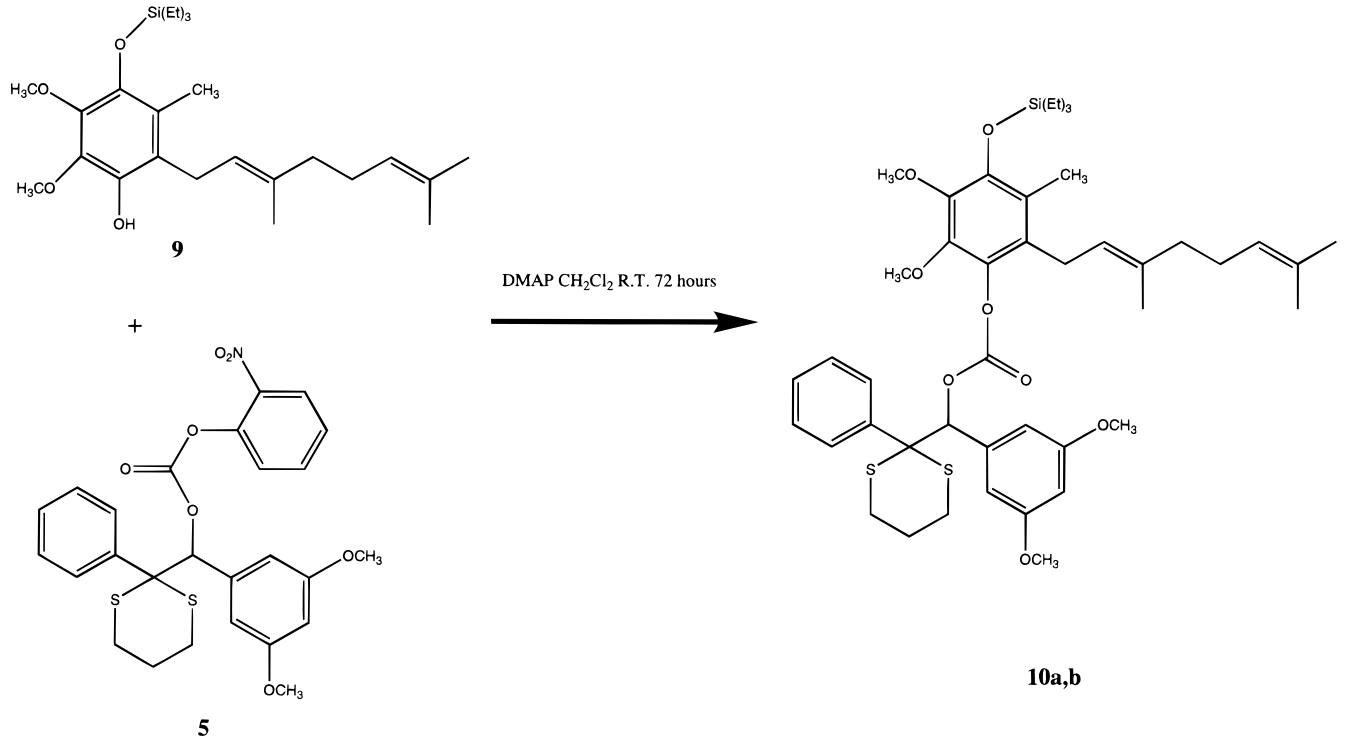
Photolysis of the Assembled Caged Ubiquinol-2. Three experimental criteria were utilized to determine the usefulness of compound **1a,b** for rapid kinetic studies. The first was to demonstrate the facile steady-state photolysis in the presence of 355 nm light. Figure 1 shows the steady-state photolysis of compound **1a,b.** Over the course of the photolysis experiment, a clean transition for the starting material to products is observed. The increase in absorbance at 300 nm is due to the formation of BF (**13**). The isobestic point at 258 nm indicates that the photolysis occurs as a single transition from the starting material to products.

Scheme 7



2 **9**

Scheme 8



9 **5** **10a,b**

Concomitant with the spectral changes observed is the formation of the desired ubiquinol-2 and the expected BF (**13**). Figure 2 shows a reverse phase HPLC trace of a number of standards as well as the starting compound **1a,b**. As can be seen, ubiquinol-2, ubiquinone-2 (**2**), and BF (**13**) are the sole products from photolysis. The presence of ubiquinone-2 is most likely due to a small amount of oxidation that occurs during handling of the sample prior to HPLC analysis. It should be noted that the extinction coefficient for ubiquinol-2 is greater than an order of magnitude (∼12×) less than that of ubiquinone-2 at the detection wavelength of 284 nm so that the molar ratio of ubiquinol-2 to ubiquinone-2 is much greater that 10:1 for the HPLC trace shown in Figure 2.

Figure 3 shows a difference chromatogram between the starting material and the photolysis product. It is evident from these data that the photolysis occurs in an exceptionally smooth manner without the production of any side products. The formation of ubiquinol-2 was further confirmed by allowing a photolyzed sample to air oxidize. HPLC analysis of this sample revealed that virtually all the ubiquinol-2 had undergone oxidation to ubiquinone-2. Figure 4 shows the results of this experiment.

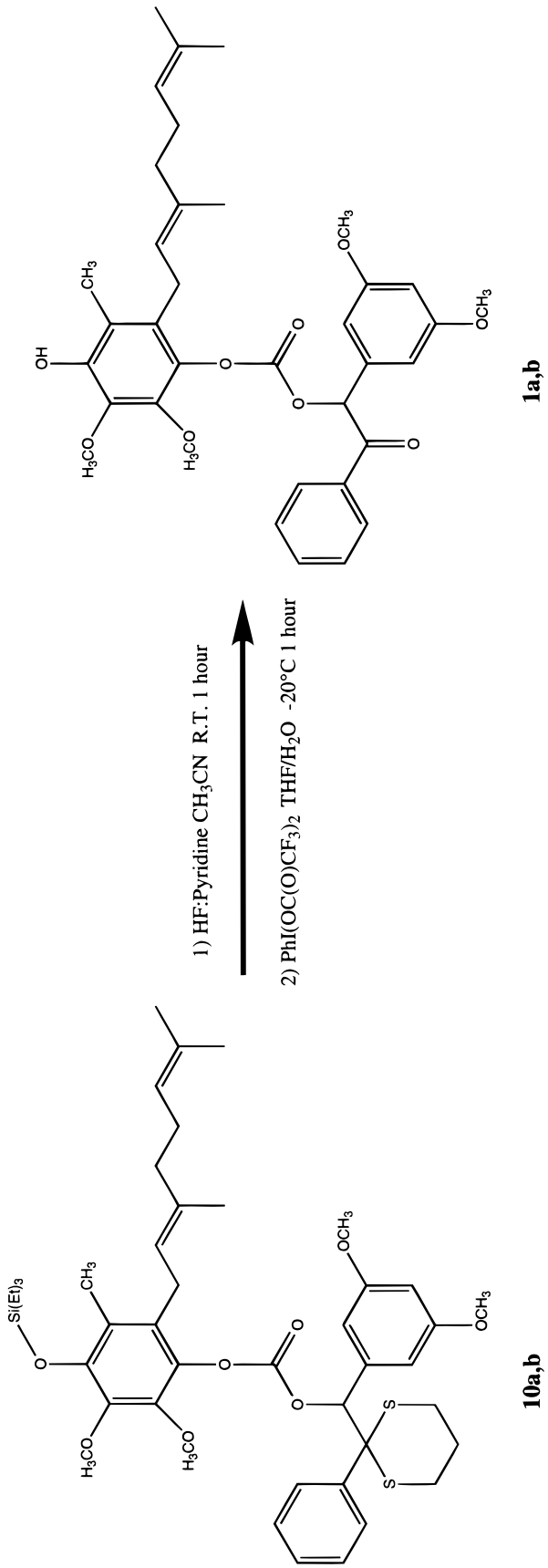
To determine the rate of photolysis, transient absorption experiments were performed to determine the rate of formation of the BF product (data not shown). These studies demonstrated that the BF photoproduct is formed within the dead time of the instrument, placing a lower bound limit on the photolysis rate of 10^6 s^{-1}.

Summary

The design and synthesis of a novel compound for studying rapid electron transfer reactions in ubiquinol oxidizing enzymes has been achieved. The photolysis rates and products are consistent with the rapid release of ubiquinol-2 on the subnanosecond time scale. It is now feasible to pursue the study of rapid electron transfer kinetics in a variety of ubiquinol oxidizing enzymes using this compound, and such studies are currently underway in our laboratory. In addition, the methods developed in this work are readily generalizable such that a variety of compounds could be similarly caged for the ultimate purpose of studying rapid kinetic events in a variety of enzymatic systems.

Experimental Section

General Methods. Anhydrous THF was prepared by refluxing over sodium metal and benzophenone. Anhydrous acetonitrile, methylene chloride, and pyridine were purchased from Aldrich. All other solvents were of reagent grade. Geranyl bromide and 2,3-dimethoxy-5-methylbenzoquinone were from Fluka. *o*-Nitrophenyl chloroformate



Scheme 9

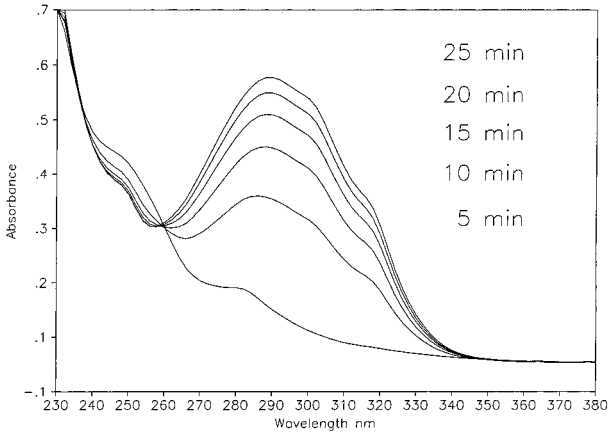


Figure 1. Steady-state photolysis of compound **1a**,**b** in methanol. See methods for details.

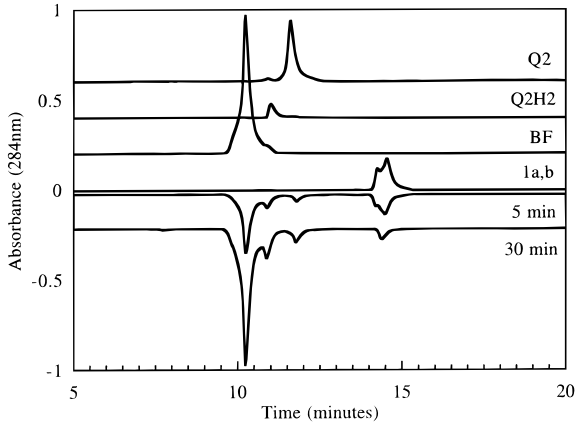


Figure 2. HPLC analysis of the photoproducts from the steady-state photolysis of compound **1a**,**b**. See methods for details. Standards are labeled, and the photoproduct samples are inverted. Q2, ubiquinone-2; Q2H2, ubiquinol-2; BF, 5,7-dimethoxy-2-phenylbenzofuran.

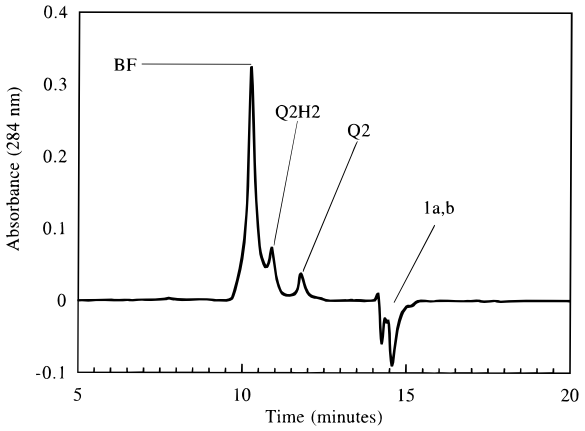


Figure 3. HPLC difference chromatogram of the 30 min photolysis products minus the starting material **1a**,**b**.

was purchased from Carbolabs, Inc. DMAP was purchased from ACROS. NMR spectra were recorded on a Bruker AM500 spectrophotometer or a GE QE300 spectrometer operating at nominal frequencies of 500 and 300 MHz for ^{1}H, respectively. High-resolution mass spectra were recorded on a Fisons VG mass spectrometer operating in FAB mode. Routine GC/MS data were recorded on a HP 5890A/5970 GC/MS equipped with a 12 m silicon gum capillary column. TLC plates were visualized with either UV light or iodine.

Steady-State Photolysis of 1. A 10 μM solution of **1** in methanol was prepared in a 1 cm path length quartz cuvette equipped with a septum. The sample was purged with oxygen free argon and irradiated

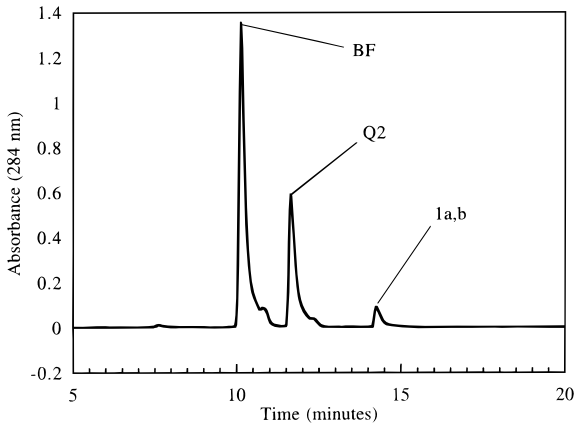


Figure 4. HPLC analysis of the photoproducts formed in Figure 2, following air oxidation.

with an Oriel 66011 Hg vapor lamp operating at 450 W and filtered through a 355 nm band-pass filter. The sample was removed, and the optical absorbance recorded at 5 min time intervals using an HP 8452 diode array spectrophotometer. HPLC analysis was performed using a Waters 625LC system equipped with a Delta-Pak C18 reverse phase.

Transient Photolysis of 1. A 10 μM solution of **1** in methanol was prepared in a 1 cm path length quartz cuvette equipped with a septum. The sample was purged with oxygen free argon and irradiated at 355 nm using a 10 ns pulse from a Nd:YAG laser. Absorbance spectra were then recorded after successive laser shots.

X-ray Crystallograpy of (±)-2,6-Dimethoxyphenoxy-3,5-dimethoxyphenyl(2-phenyl-1,3-dithi-2-yl)methoxymethanone (6). A total of 10 078 reflections were collected at room temperature using a CAD-4 diffractometer and the ω-scan method. The data were reduced and merged in CRYM (Duchamp, D. J. *Amer. Crystallogr. Assoc. Meet.* **1964**, 29) to give a total of 10 078 unique reflections with an R_{cryst} 0.042. Structure solution was performed with SHELXS-86 direct methods (Sheldrick, G. M. *Acta. Crystallogr*. **1990**, *A46*, 467). The model was refined against F^2 using $1/\sigma^2$ weights with SHELXL-93 (Sheldrick, G. M., University of Gottingen), and the positions of hydrogen atoms were revealed in difference Fourier maps.

Synthesis of 4,5-Dimethoxy-2-methyltricyclo[6.2.1.0^{2,7}]undeca-4,9-diene-3,6-dione (7). A 25 mL flask was charged with a stir bar, 5 mL of dry methanol, and 500 mg (3 mM) of 2,3-dimethoxy-5-methyl-1,4-benzoquinone. To this solution is added 3 mL of freshly distilled cyclopentadiene. The reaction is allowed to stir overnight during which time the dark reddish orange color of the 2,3-dimethoxy-5-methyl-1,4-benzoquinone fades to the pale yellow color of the product. Solvent and excess cyclopentadiene are removed in vacuo to give a pale yellow oil. Yield 0.75 g, (>99%). ^{1}H NMR ($CDCl_3$, TMS) δ 6.15 (m, 1H), 6.00 (m, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.41 (bs, 1H), 3.07 (bs, 1H), 2.82 (d, 1H) 1.66 (d, 1H), 1.53 (d, 1H), 1.47 (s, 3H); HRMS (FAB) *m*/*z* (MH^+) calcd 249.11267, obsd 249.11283.

Synthesis of 2-[3,7-Dimethyl-(2*E*)-2,6-octadienyl]-4,5-dimethoxy-7-methyltricyclo[6.2.1.0^{2,7}]undeca-4,9-diene-3,6-dione (8). A flame dried flask is charged with geranyl bromide (1.44 g, 6.6 mmol), **7** (1.5 g, 6 mmol), a stir bar, and 40 mL of dry 1/3 toluene/*tert*-butyl alcohol. This solution is cooled to 0 °C using an ice bath, and 10 mL of 1 M potassium *tert*-butoxide in *tert*-butyl alcohol is added via cannula with rapid stirring. The solution immediately turns a dark brown color, and the reaction is allowed to stir for 1 h at 0 °C. The reaction mixture is then poured into 50 mL of saturated NH_4Cl and extracted with 2 × 100 mL of dry diethyl ether. The organic layers are combined, filtered through a plug of $MgSO_4$, and solvent removed in vacuo. The resultant dark brown oil is purified by flash column chromatography using 4/1 hexane ethyl acetate to yield 1.68 g (73%) of **8**. ^{1}H NMR ($CDCl_3$, TMS) δ 6.05 (bs, 2H), 5.07 (m, 2H), 3.90 (s, 3H), 3.88 (s, 3H), 3.05 (d, 2H), 3.75 (dd, 1H), 2.42 (dd, 1H), 2.00 (m, 4H), 1.65 (s, 3H), 1.57 (s, 3H), 1.58 (s, 3H), 1.49 (s, 3H), 1.78 (d, 1H), 1.45 (d, 1H); HRMS (FAB) (MH^+) *m*/*z* calcd 384.230060, obsd 384.228649.

Synthesis of 2-[3,7-Dimethyl-(2*E*)-2,6-octadienyl]-5,6-dimethoxy-3-methylbenzo-1,4-quinone (Ubiquinone-2) (2). A stirred solution of **8** (1.68 g) dissolved in 20 mL of toluene was refluxed for 60 min; during this time the solution turned from a pale yellow to a reddish orange. Solvent was removed in vacuo and the resultant oil purified by flash column chromatography using 4/1 hexane/EtOAc. Yield 1.4 g (>99%). ^{1}H NMR ($CDCl_3$, TMS) δ 5.03 (t,1H), 4.93 (t, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 3.18 (d, 2H), 2.01 (s, 3H), 1.99 (m, 4H), 1.73 (s, 3H), 1.65 (s, 3H), 1.58 (s, 3H); HRMS (FAB) *m*/*z* (MH^+) calcd 319.19876, obsd 319.199838.

Synthesis of 4-(Triethylsiloxy)-2-[3,7-dimethyl-(2*E*)-2,6-octadienyl]-5,6-dimethoxy-3-methylphenol and 4-(Triethylsiloxy)-3-[3,7-dimethyl-(2*E*)-2,6-octadienyl]-5,6-dimethoxy-2-methylphenol (9a and 9b). A round-bottom flask equipped with stir bar is charged with **2** (0.63 g, 2.0 mmol) and 10 mL of 10/1 methanol/water. With vigorous stirring, sodium dithionite (0.38 g, 2.2 mmol) was added. The reddish orange color of the ubiquinone-2 quickly dissipates, signaling the formation of the ubiquinol. The resultant slurry is extracted with 100 mL of hexane and filtered into a dry round-bottom flask through a small plug of $MgSO_4$. Solvent is removed in vacuo, and the nearly colorless oil is redissolved in dry acetonitrile and the flask purged with dry nitrogen. The flask is charged with a stirring bar and dry pyridine (0.16 g, 2.2 mmol). Chlorotriethylsilane (0.33 g, 2.2 mmol) was added dropwise using a syringe pump over a 6 h period. The reaction was monitored by GC/MS. Solvent and excess TESCl were removed in vacuo when the disilyl product began to accumulate. The resultant oil is purified by flash chromatography using 9/1 hexane/EtOAc to give a clear colorless oil. Yield 0.4 g (61%). ^{1}H NMR ($CDCl_3$, TMS) for **9a** δ 5.43 (s, 1H), 5.19 (m, 2H), 3.89 (s, 3H), 3.76 (s, 3H), 3.28 (d, 2H), 2.09 (s, 3H), 2.00 (m, 4H), 1.72 (s, 3H), 1.65 (s, 3H), 1.56 (s, 3H), 0.95 (t, 9H), 0.73 (q, 6H). ^{1}H NMR ($CDCl_3$, TMS) for **9b** δ 5.41 (s, 1H), 5.06 (m, 2H), 3.89 (s, 3H), 3.76 (s, 3H), 3.31 (d, 2H), 2.09 (s, 3H), 2.00 (m, 4H), 1.75 (s, 3H), 1.65 (s, 3H), 1.56 (s, 3H), 0.96 (t, 9H), 0.73 (q, 6H); HRMS (FAB) *m*/*z* (MH^+) calcd 435.29304, obsd 435.29323 for a mixture of **9a** and **9b**.

Synthesis of (±)-3,5-Dimethoxyphenyl-2-phenyl-1,3-dithi-2-yl-methanol (3). A flame dried flask was charged with a stir bar, 100 mL of dry THF, and 2-phenyl-1,3-dithiane (3.9 g, 20 mmol). This solution is cooled to 0 °C, and 22 mmol of *n*-butyllithium was added dropwise via syringe (11 mL of a 2.0 M solution in hexane). Formation of the dithiane anion is indicated by the presence of a pale olive green color. Upon completion of the addition the reaction is allowed to stir for 30 min, and then 3,5-dimethoxy benzaldehyde dissolved in dry THF is added via syringe (3.3 g, 20 mmol, 2 × 10 mL THF). The reaction is allowed to stir for 1 h at 0 °C and then poured into 100 mL of 0.1 M HCl and extracted with diethyl ether. The organic phase was filtered through a plug of $MgSO_4$, and solvent removed in vacuo to yield a colorless oil which was further purified by flash chromatography using 4/1 hexane/EtOAc. Yield: 7.1 g (98.6%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (dd, 2H), 7.31 (m, 3H), 6.30 (t, 1H), 5.99 (d, 2H), 4.94 (d, 1H), 3.00 (d, 1H), 3.57 (s, 6H), 2.74−2.65 (bm, 4H), 1.94−1.90 (bm, 2H); HRMS (FAB) *m*/*z* (MH^+) calcd 363.1122, obsd 363.1513.

Synthesis of (±)-2-Nitrophenoxy-3,5-dimethoxyphenyl(2-phenyl-1,3-dithi-2-yl)methoxymethanone (5). A flame dried flask equipped with stirring bar and septum was charged with **3** (8.16 g, 24.4 mmol), 150 mL of dry methylene chloride, and 2-nitrophenyl chloroformate at room temperature under dry nitrogen. To this solution was added 2.2 equiv of dry pyridine via syringe. The solution was allowed to stir for 1 h at room temperature, and then solvent was removed in vacuo. The resultant residue was washed with dry ether, and the ether extract was filtered through a plug of $MgSO_4$. Solvent was removed in vacuo to yield a thick oil which when dried under high vacuum produced a pale yellow solid. Yield 9.96 g (77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (dd, 1H), 7.74 (m, 2H), 7.66 (t, 1H), 7.42 (t, 1H), 7.32 (m, 4H), 6.36 (t, 1H), 6.03 (s, 1H), 6.01 (d, 2H), 3.59 (s, 6H), 2.75 (m, 4H), 1.96 (m, 2H); HRMS (FAB) *m*/*z* (MH^+) cacld 527.107246, obsd 527.106851.

Synthesis of (±)-2,6-Dimethoxyphenoxy-3,5-dimethoxyphenyl(2-phenyl-1,3-dithi-2-yl)methoxymethanone (6). A flame dried flask equipped with stir bar and septum was charged with **5** (0.53 g, 1 mmol), 2,6-dimethoxyphenol (0.15 g, 1 mmol), DMAP (0.13 g, 1 mmol), and 10 mL of methylene chloride under dry nitrogen. The reaction was allowed to stir at room temperature for 72 h, then poured into 10 mL

of 0.1 M HCl, and extracted with 2 × 10 mL of methylene chloride. The organic phases were combined and filtered through a plug of $MgSO_4$ and solvent removed in vacuo to yield a crystalline solid. Recrystallization from ethyl acetate gave large rectangular rods. Yield 0.54 g (99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (dd, 2H), 7.28 (m, 3H), 7.12 (t, 1H), 6.59 (d, 2H), 6.33 (t, 1H), 6.03 (s, 1H), 6.01 (d, 2H), 3.75 (s,6H), 3.58 (s, 6H), 2.85−2.55 (bm, 4H), 2.05−1.85 (bm, 2H); HRMS (FAB) *m*/*z* (M^+) calcd 542.143297, obsd 542.143499. Unit cell, $a = 9.201$, $b = 28.599$, $c = 10.664$, $\alpha = 90.0$, $\beta = 104.05$, $\gamma = 90.0$. Space group $P2(1)/c$. Reflections 10 078, R_{cryst} 0.042.

Synthesis of (±)-4-(Triethylsiloxy)-2-[3,7-dimethyl-(2*E*)-2,6-octadienyl]-5,6-dimethoxy-3-methylphenoxy-3,5-dimethoxyphenyl(2-phenyl-1,3-dithi-2-yl)methoxymethanone and 4-(Triethylsiloxy)-3-[3,7-dimethyl-(2*E*)-2,6-octadienyl]-5,6-dimethoxy-2-methylphenoxy-3,5-dimethoxyphenyl(2-phenyl-1,3-dithi-2-yl)methoxymethanone (10a and 10b). A flame dried flask equipped with stir bar and septum was charged **5** (1.73 g, 4 mmol), **9a,b** (2.2 g, 4.2 mmol), DMAP (0.5 g, 4.1 mmol), and 50 mL of dry methylene chloride under dry nitrogen. The reaction was allowed to stir for 72 h and then poured into 25 mL of 0.1 M HCl and extracted with 2 × 50 mL of methylene chloride. The organic phases were combined and filtered through a plug of $MgSO_4$ and solvent removed in vacuo. The resultant oil was purified by flash chromatography using 5/1 hexane/EtOAc. Yield 1.8 g (55%). R_f = 0.5 (5/1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$, TMS) δ 7.78−7.65 (m, 2H), 7.35−7.25 (m, 3H), 6.35 (t, 1H), 6.05 (d, 2H) 5.9 (s, 1H), 5.10−4.86 (bm, 2H), 3.72 (s, 3H), 3.68 (s, 3H), 3.59 (s, 6H), 3.38−3.10 (bm, 2H), 2.85−2.60 (bm, 4H), 2.18−1.84 (bm, 9H), 1.64 (m, 3H), 1.54 (m, 6H), 0.96 (m, 9H), 0.76 (m, 6H); HRMS (FAB) *m*/*z* (MH^+) calcd 823.375702, obsd 823.373367.

Synthesis of (±)-3,5-Dimethoxyphenyl(2-phenyl-1,3-dithi-2-yl)-methoxy-2-[3,7-dimethyl-2(*E*)-2,6-octadienyl]4-hydroxy-5,6-dimethoxy-3-methylphenoxymethanone and (±)-3,5-Dimethoxyphenyl(2-phenyl-1,3-dithi-2-yl)methoxy-3-[3,7-dimethyl-2(*E*)-2,6-octadienyl]4-hydroxy-5,6-dimethoxy-2-methylphenoxymethanone (12a and 12b). A flask equipped with a stir bar was charged with 10 mL of acetonitrile and 0.5 g (0.6 mmol) of **10a,b**. With rapid stirring several drops of pyridinium hydrogen fluoride was added. The reaction was monitored by TLC and solvent removed in vacuo when all the starting material had been converted to a lower R_f product. The resultant solid was washed with dry ether (3 × 25 mL), and the ether extracts were combined, filtered through a plug of silica/$MgSO_4$, and solvent removed in vacuo to yield a white solid. Yield 0.43 g (99%). ^{1}H NMR ($CDCl_3$, TMS) δ 7.78−7.65 (m, 2H), 7.35−7.25 (m, 3H), 6.35 (t, 1H), 6.05 (d, 2H) 5.9 (s, 1H), 5.10−4.86 (bm, 2H), 3.72 (s, 3H), 3.68 (s, 3H), 3.59 (s, 6H), 3.38−3.10 (bm, 2H), 2.85−2.60 (bm, 4H), 2.18−1.84 (bm, 9H), 1.64 (m, 3H), 1.54 (m, 6H); HRMS (FAB) *m*/*z* (M^+) calcd. 708.279063, obsd 708.274750.

Synthesis of (±)-3,5-Dimethoxyphenyl-2-{2-[3,7-dimethyl-2(*E*)-2,6-octadienyl]-4-hydroxy-5,6-dimethoxy-3-methylphenoxycarbonyloxy}-1-phenyl-1-ethanone and (±)-3,5-Dimethoxyphenyl-3-{2-[3,7-dimethyl-2(*E*)-2,6-octadienyl]-4-hydroxy-5,6-dimethoxy-2-methylphenoxycarbonyloxy}-1-phenyl-1-ethanone (1a and 1b). A flask equipped with a stir bar was charged with **12a,b** (0.5 g, 0.6 mmol) and 10 mL of 10/1 THF/water and cooled to −20 °C with an ethylene glycol dry ice bath. To this solution was added bis(trifluoroacetoxy)-iodobenzene (0.26 g, 0.6 mmol) dissolved in 1 mL of THF dropwise. After completion of the addition the solution was stirred for 30 min and then allowed to warm to room temperature. Solvent was removed in vacuo, the resultant oil extracted with dry ether (3 × 25 mL), and the ether extracts were combined and filtered through a plug of silica/$MgSO_4$, and solvent was removed in vacuo. The resultant oil was purified by flash chromatography using 11/1 hexane/EtOAc. Yield 0.083 g (22%). ^{1}H NMR ($CDCl_3$, TMS) δ 7.78−7.65 (m, 2H), 7.35−7.25 (m, 3H), 6.35 (t, 1H), 6.05 (d, 2H) 5.9 (s, 1H), 5.10−4.86 (bm, 2H), 3.72 (s, 3H), 3.68 (s, 3H), 3.59 (s, 6H), 3.38−3.10 (bm, 2H), 2.18−1.84 (bm, 7H), 1.64 (m, 3H), 1.54 (m, 6H); HRMS *m*/*z* (MH^+) calcd 619.290708, obsd. 619.290763

Acknowledgment. The authors would like to thank Ronald S. Rock for synthetic advice and Theodore J. DiMagno for assistance with the transient absorption studies. This work was supported in part by NIH Grant GM 22432 from the National Institute of General Medical Sciences, U.S. Public Health Service.

Supporting Information Available: ORTEP drawing of compound **6** (1 page). See any current masthead page for ordering and Web access instructions.

JA971079J