Design and Synthesis of Photoreleasable Ubiquinol and Its Biologically Active Analogues

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Abstract

Every organism contains a respiratory chain that converts energy from food molecules into adenosine triphosphate (ATP) which drives a multitude of biochemical reactions. A respiratory chain achieves this via a series of integral membrane protein complexes that produce a transmembrane proton gradient as a result of sequential electron transfers through these complexes. Ubiquinol and ubiquinol oxidase enzymes are important components of the respiratory chains in most aerobic organisms. The energy from the oxidation of ubiquinol is used to promote the generation of an electrochemical gradient across the cytoplasmic or mitochondrial membrane for ATP synthesis.

Study of the catalytic mechanisms of ubiquinol oxidase enzymes is very difficult due to the structural complexity of these proteins. Since electron input from ubiquinol plays a very important role in enzyme function, to understand the detailed enzymatic mechanism of these enzymes, it is critical to understand the kinetics of individual electron transfer events in enzymatic turnover. On account of the rapidity of electron transfer in these proteins, traditional stopped-flow methods for following the kinetic course of electron transfer reactions are limited, due to the millisecond order of mixing dead time.

Photochemical initiation of ubiquinol release, a method of circumventing the mixing limitation, is investigated. The method is based on the photolysis of small organic protecting groups, or "cage" compounds. These cage compounds rendered

inactive quinol-cage complex prior to photolysis by linking to the key functional groups in ubiquinol or by generating a large steric hindrance that blocks ubiquinol binding. Photolysis of the cage-quinol complex rapidly releases the two-electron donor and triggers on the enzymatic electron transfer. Such a complex can be used not only in enzymatic electron transfer study, but also in time-resolved protein conformation change study.

The cage compounds that have been studied for this purpose are based on 3', 5'-dimethoxybenzoin (DMB). Esters of DMB are known to photolyze rapidly (on the sub-nanosecond time scale), generating the parent acid and the inert photoproduct 5,7-dimethoxy-2-phenylbenzofuran. A water-soluble derivative of 3', 5'-dimethoxybenzoin (DMB), 3', 5'-bis (carboxylmethoxy)benzoin (BCMB) was linked to the hydroxy group in ubiquinol via a carbonate linkage. Such a complex has been measured to yield a photorelease rate of $990s^{-1}$ in detergent solution. Another cage compound, N-hydroxypyridine-2-thione, was also used to "cage" ubiquinol via a carbonate linkage. In the presence of efficient hydrogen donor, such as mercaptoethanol, such a complex has the potential to photolytically generate ubiquinol in submillisecond time scale.

A series of ubiquinol analogues were synthesized with a carboxylic acid functionality attached to benzoquinone at different positions. These analogues can be "caged" by BCMB via an ester bond with the potential to trigger electron transfer in sub-microsecond.

In the mean time, efforts have been made to elucidate the controversial photolysis mechanisms of benzoin compounds by synthetic substitution study and quantum

chemistry calculation. Very efficient synthetic schemes for the preparation of large-size combinatorial libraries of benzoins were developed.

Table of Contents

Abbreviations and Nomenclature

ATP	Adenosine triphosphate
BCMB	3',5'-Bis(carboxymethoxy)benzoin
CAN	Ceric Ammonium Nitrate
CM-PEG	Carboxymethyl-Poly(ethylene glycol)
CO	Carbon monoxide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	N,N-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIEA	N,N-Diisopropylethylamine
DMAP	N,N-Dimethylaminopyridine
DMB	3',5'-Dimethoxybenzoin
DMF	N,N-Dimethylformamide
DMNB	4,5-Dimethoxy-2-nitrobenzyl
DNP	3,5-Nitrophenyl
EDT	1,2-Ethanedithiol
FAB-MS	Fast atom bombardment mass spectrometry
HTOC	N-hydroxy-2-thiopyridone oxycarbonyl
IR	Infrared
ISP	Iron-sulfur Protein
MPP	Mitochondrial Processing Peptidase
MS	Mass Spectrometry
NB	2-nitrobenzyl
NPE	1-(2-nitrophenyl)ethyl
OCO	Oxycarbonyl
OSS	Open Shell Singlet
PDS	Pyridium dichromate
PEG	Poly(ethylene glycol)
PRC	Photosynthetic Reaction Center
PTC	Phase Transfer Condition
TBAF	Tetrabutylammonium fluoride
TBDMS	Tert-butyldimethylsilyl
TEBA	Triethylbenzylammonium chloride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIS	Triisopropylsilane
TMEDA	Tetramethylethylenediamine

Chapter 1

Ubiquinol and Ubiquinol Oxidase Enzymes

Ubiquinol

Ubiquinol (2,3–dimethoxy–5–methyl–6–polyprenyl-1,4–hydro-benzo-quinone) (Figure 1.1) is an important component of the mitochondrial respiratory chain, mediating electron transfer between NADH and succinate dehydrogenase and the cytochrome system. The side chain in the 6-position of the benzoquinone ring ranges from 6 to 10 isoprenoid units, being Q_6 common in yeast, CoQ_8 in some bacteria, and CoQ_9 and CoQ_{10} in vertebrates[18]. It functions as an electron and proton carrier in mitochondria and bacterial electron transport coupled to ATP synthesis. In addition, as the only known lipid-soluble antioxidant that mammalian cells can synthesize de novo, ubiquinol also acts as an antioxidant, inhibiting lipid peroxidation in biological membranes and in serum low-density lipoprotein (LDL). Recent studies indicate that it can also protect mitochondrial inner-membrane proteins and DNA against oxidative damage[16, 3, 4, 5].

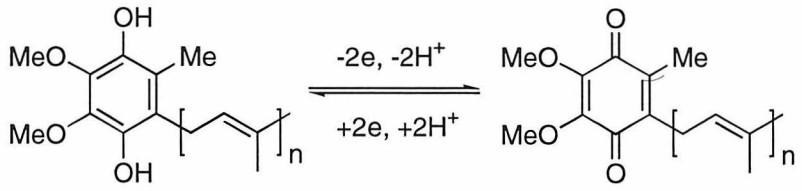


Figure 1.1. Ubiquinol - a Two-Electron Donor

In mammalian cells, the biosynthesis of ubiquinol and ubiquinone, the oxidative form of ubiquinol, is the result of two metabolic sequences. The quinone moiety is derived predominantly from tyrosine (in some instances from phenylalanine) which is converted through a number of steps to 4-hydroxybenzoate. The polyprenyl side chain is

synthesized from acetyl-CoA through the mevalonate pathway, which leads to the formation of farnesyl-PP. After converted to decaprenyl-PP (or in rodents solanesyl-PP), the farnesyl-PP condenses with 4-hydroxybenzoic acid to form decaprenoyl-(or nonaprenoyl)-4-hydroxybenzoate. Through a number of sequential reactions, the decaprenoyl-(or nonaprenoyl)-4-hydroxybenzoate is then converted to ubiquinone. Within the cell, besides mitochondria, ubiquinone and ubiquinol also occur in the endoplasmic reticulum, the Golgi apparatus, the lysosomes, the peroxisomes and the plasma membrane[28, 23].

Ubiquinol and ubiquinone are widely located in animal cells, tissues and blood. In various human and rat tissues, ubiquinone is present partly in the ubiquinol form, ranging from 20% to 100%[1]. In blood, where it is bound to serum lipoproteins[23], ubiquinone also occurs partly as ubiquinol with antioxidant functions[36]. Tissue ubiquinonol levels are regulated by physiological factors related to the oxidative activity of the organism[3,17]: they increase under the influence oxidative stress and decrease during aging.

Respiratory Chain

Every organism contains a respiratory chain that converts the energy from food molecules into adenosine triphosphate (ATP) through multiple biochemical pathways. A typical respiratory chain consists of a series of oligomeric, cytochrome-containing integral membrane protein complexes that are inserted into the inner mitochondrial membrane or bacterial plasma membrane. These respiratory enzymes convert the free energy from oxidation into a transmembrane proton electron gradient as a result of

sequential electron transfers. This gradient consists of two components, a proton concentration gradient and a transmembrane electrical potential. This gradient provides the electrochemical potential for energy-requiring reactions such as synthesis of ATP, cell motility, transport of ions and metabolites.

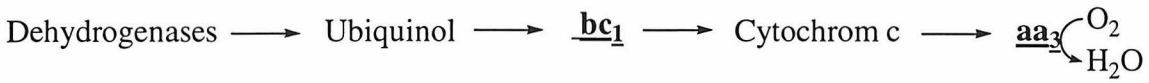
In the oxygenic respiratory process, oxygen is the terminal electron acceptor. Two types of cytochrome complexes, the cytochrome bc_1 complex and the terminal oxidases, participate in respiration, and link electron transfer to proton translocation. The cytochrome bc_1 does not transfer electrons to oxygen, whereas the terminal oxidases do. The relationship between the cytochrome complexes in the electron transfer chains of mitochondria and two representative bacteria are shown in the Figure 1.2. It is obvious in Figure 1.2 that ubiquinol is a very important component of respiratory chains. It functions as a confluent point for the collection of reducing equivalents from dehydrogenases and linking metabolism to cellular energetics.

In mitochondria of most species, oxidation of ubiquinol by molecular oxygen is catalyzed by the sequential action of two enzyme complexes: the cytochrome bc_1 complex, which oxidizes quinol and reduces cytochrome c, and the cytochrome c oxidase complex, which oxidizes cytochrome c and reduces oxygen to water, as shown in Figure 1.2A.

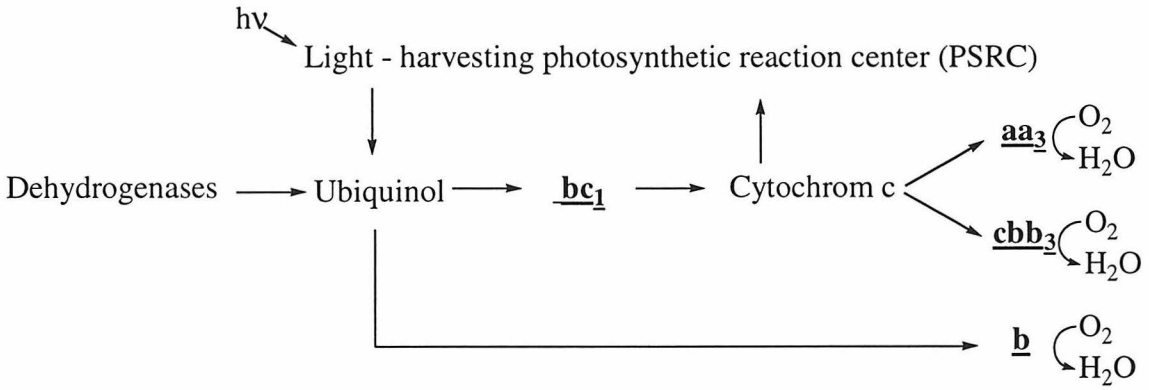
Bacteria that oxidize quinol through a cytochrome bc_1 complex possess at least one alternate quinol oxidase, which transfers electrons directly from the quinol to oxygen. As shown in Figure 1.2B, there exists a quinol oxidase that oxidizes ubiquinol without the intermediate participation of the bc_1 complex. In addition to participating in

respiration, the cytochrome bc_1 complex also participates in light-driven cyclic electron transfer when photosynthetic bacteria such as *Rhodobacter Sphaeroides* grow phototrophically.

(A) In Mitochondria



(B) In Purple Nonsulfur Photosynthetic Bacteria



(C) In *E.Coli*

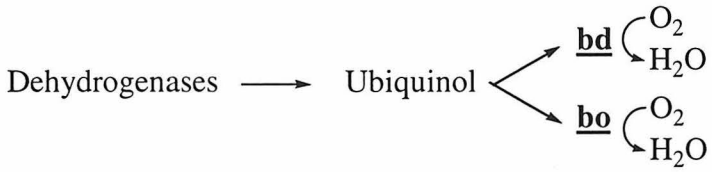


Figure 1.2. Energy Transducing Proteins in Mitochondria and Bacterial Respiration

The simplest arrangement of respiratory cytochrome complexes is illustrated by *Escherichia Coli*, which lacks a cytochrome bc_1 complex and oxidizes quinol directly with molecular oxygen by either of the two quinol oxidases, as illustrated in Figure 1.2C.

A question of fundamental interest is how the electron transfer reactions catalyzed by the cytochrome complexes are coupled to the generation of a transmembrane pH gradient. In our research laboratory, we have focused our investigations on the two respiratory cytochrome complexes, cytochrome bc_1 and cytochrome bo_3. The enzymology and structure characteristics of these two enzymes are briefly described below.

Cytochrome bc_1 Complex

Ubihydroquinone:cytochrome c oxidoreductase (cytochrome bc_1, Complex III) is an integral membrane protein that forms the middle part of the respiratory chain in eukaryotes and many prokaryotes[20, 29]. The reaction catalyzed by the bc_1 complex is described by the equation 1, in which the subscripts "i" and "o" designate the inner (negative) and outer (positive) sides of the membrane, respectively.

$$QH_2 + 2\ \text{cyt}\ c_{ox} + 2\ H^+_i \rightarrow Q + 2\ \text{cyt}\ c_{red} + 4\ H^+_o \quad (1)$$

The bc_1 complex is a member of the family of bc-type complexes, which includes the b*f* cytochromes that are found in chloroplasts and some bacteria. All bc_1 complexes contain four redox prosthetic groups: a di-heme cytochrome b, cytochrome c_1, and a high

potential iron-sulfur cluster[30, 35]. No bc_1 complex is known to contain any additional redox prosthetic groups. The mammalian enzyme is composed of eleven protein subunits, three of which are associated with the four redox centers. The rest are non-redox active proteins, the so-called supernumeral subunits. Their role in the complex has long been assumed to be structural rather than catalytic. Recent studies discovered that plant mitochondrial bc_1 complexes from wheat, potato and spinach have mitochondrial processing peptidase (MPP) activity[11,10] in addition to electron transfer activity. However, no similar peptidase activity is detected in the bovine bc_1 complex.

The cytochrome bc_1 complex spans the inner mitochondrial membrane as illustrated in Figure 1.3. Cytochrome b is a transmembrane protein in which the two b heme groups form an electrical circuit between the two ubiquinol/ubiquinone binding sites that are on each side of the membrane. The low potential b heme (b_L, or b_{566}) resides near the positive side of the membrane and the high potential heme (b_H, or b_{562}) resides near the center of the membrane. The Iron-sulfur protein (ISP) is anchored to the complex by a hydrophobic helix at the amino terminus of the protein[22]. Cytochrome c_1 is anchored to the membrane by a hydrophobic helix at its C-terminus. The domain that contains the heme is accessible, where cytochrome bc_1 reacts with cytochrome c.

Electron transfer through cytochrome bc_1 is coupled to transmembrane proton translocation with a stoichiometry of $H^+/e^- = 2$ and to the translocation of a single positive charge (*i.e.*, $q^+/e^- = 1$) [34]. The mechanisms of electron transfer and proton translocation by cytochrome bc_1 are still under debate. A widely accepted and long-standing mechanism is known as the Q-cycle, which was originally proposed by Mitchell

[25, 32]. The key feature of the Q-cycle hypothesis is that there are two separate binding sites for ubiquinol and ubiquinone.

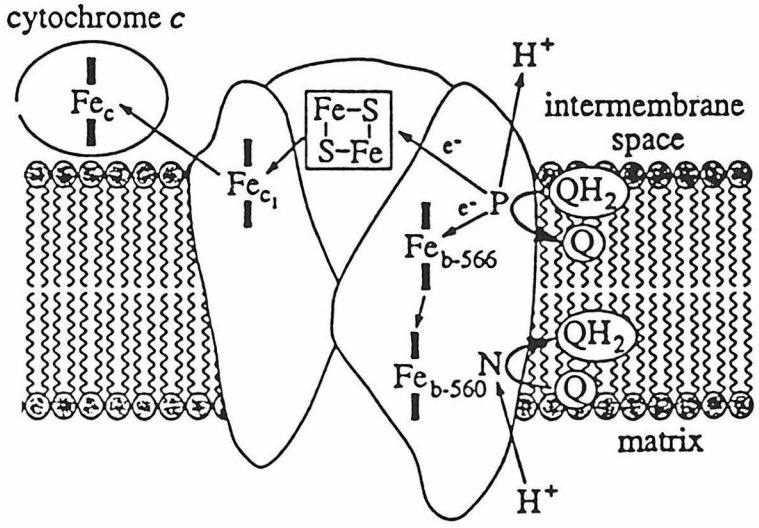


Figure 1.3. Cytochrome bc_1 Complex

Ubiquinol is oxidized at the Q_O site near the positive side of the inner mitochondrial membrane and ubiquinone is reduced at the Q_i site near the negative side of the membrane. In Q-cycle hypothesis, there are two electron transfer pathways in cytochrome bc_1. One is the high-potential pathway composed of the Fe-S protein ($E_{m,7}$ = 300 mV) and cytochrome c_1 ($E_{m,7}$ = 250 mV), and the other one is a low-potential electron transfer pathway composed of cytochrome b_{566} ($E_{m,7}$ = -50 mV) and cytochrome b_{562} ($E_{m,7}$ = 50 mV). Ubiquinol donates one electron to the high potential Fe-S protein

and this electron is then passed onto cytochrome c_1. The second electron from ubiquinol takes the low potential path to a quinone bound on the positive side of the membrane, forming semiquinone. The cycle is completed when the above steps are repeated. This time, as the second electron passes down the low potential path, the semiquinone is reduced to a quinol, concomitantly taking two protons up from the matrix side of the membrane. During one complete Q cycle, two molecules of ubiquinol are oxidized to ubiquinone and one molecule of ubiquinone is reduced to ubiquinol in two steps.

Crystal structure[14] shows that the complex is a symmetric dimer, in which the closely interacting monomers form two clefts. The quinol binding pockets can be accessed through the clefts. The Q_i site of one monomer and the Q_o site of the other monomer are accessible through the same cavity, suggesting that quinone formed at the Q_i site can proceed to the Q_o site of the other monomer without leaving the bc_1 complex. The cavities seem to be tightly sealed toward the inter-membrane space, but are solvent-accessible from the matrix side. This arrangement would allow proton uptake at the Q_i site from the matrix. Unlike the Q_i site in this model, the Q_o site is not in direct contact with either the b_L heme or the Fe-S cluster. Instead, it is surrounded by aromatic residues that may mediate electron transfer.

A critical question in Q-cycle mechanism is the mandatory bifurcation of electron transfer at the Q_o site[32, 9, 6]. It is not fully understood yet why the second electron from semiquinone at the Q_o site does not reduce the ISP even under conditions that block the b-heme pathway. Various theories have been proposed to explain the electron transfer bifurcation[6, 7, 13, 15].

Berry and Kim reported two different locations for the extra-membranous domain of the ISP in the chicken bc_1 complex, suggesting that the ISP may be moving around a pivot joint during the catalytic cycle of the functioning bc_1 complex. Crystal structure and crystallographic inhibitor studies also suggested two transient quinone-binding sites in the inhibitor-binding pocket at the Q_o site. One Q binding site is closer to ISP and designated as P_1; the other Q binding site is closer to b_L heme and designated as P_2. Binding of ubiquinol to P_1 will cause ISP to be less mobile, and binding of ubiquinol to P_2 will release ISP. In a Q-cycle, a ubiquinol molecule comes in, first binds to the P_1 site, and ISP is immobilized, then one proton is released followed by transfer of one electron to ISP. After the second proton is released, the ubisemiquinone radical moves from the P_2 site, which causes the ISP to be released, and prevents the remaining electron from going to ISP. The second electron of quinol (ubisemiquinone) then reduces the b_L heme. This completes the cycle in a manner similar to that in the proposed catalytic switch model[7]. This model requires that the P_1 site has a higher affinity for ubiquinol when FeS is in the oxidized state, and that the P_2 site has a higher affinity for ubisemiquinone when b_L is in the oxidized state. The bifurcation of electron transfer at the Q_o site is therefore enforced by the large movement of the ISP. The movement is induced by the binding of different redox states of Q at two different subsites in the Q_o binding pocket.

An alternative hypothesis for the electron transfer event at the Q_o site is that instead of ubiquinol alone, the electron donor of ISP is the ubiquinol-heme-b_L complex. After the first electron of ubiquinol in the complex transfers to ISP, the second electron rapidly transfers to heme $b_L$and no semiubiquinone is generated. The electron transfer

from heme b_L to heme b_H results in a conformational change of cytochrome b protein, which makes (or allows) the reduced ISP to move to a position closer to heme c_1 to allow electron transfer to take place.

Recently, complete X-ray crystal structures of the 11-subunit bovine mitochondrial cytochrome bc_1 complex and inhibitor-bound complexes (myxothiazol and antimycin A) have been published[21]. Based on the new structural information, along with previous biochemical data, a three-state model was proposed. The features of this model are: 1) There are three positional states for ISP determined by its interactions with different ionic and redox state of ubiquinol. Electron transfers are gated by the three positional states of ISP. 2) Electron transfer reaction between species only occurs when they are hydrogen bonded to each other. 3) The rate limiting factors are the ubiquinol deprotonation[8] and the stability of the semiquinone intermediate[24].

Cytochrome bo_3 Complex

Cytochrome bo_3 is a component of the respiratory chain of *E. coli* grown under aerobic conditions, serving as the primary terminal oxidase in *E. coli* under conditions of high oxygen concentration. It is a membrane-bound enzyme with a molecular weight of 140000[12]. Cytochrome bo_3 is a member of the superfamily of heme-copper oxidases. The enzyme consists of four protein subunits which accommodate three redox-active metal centers, all bound to subunit I: a low-spin heme b and a heme – copper binuclear center (heme o_3-Cu_B). It also contains a bound ubiquinone molecule, Q_B, in close proximity to heme b[31]. Electron-donating ubiquinol binds to subunit II[33]. Dioxygen is bound to the

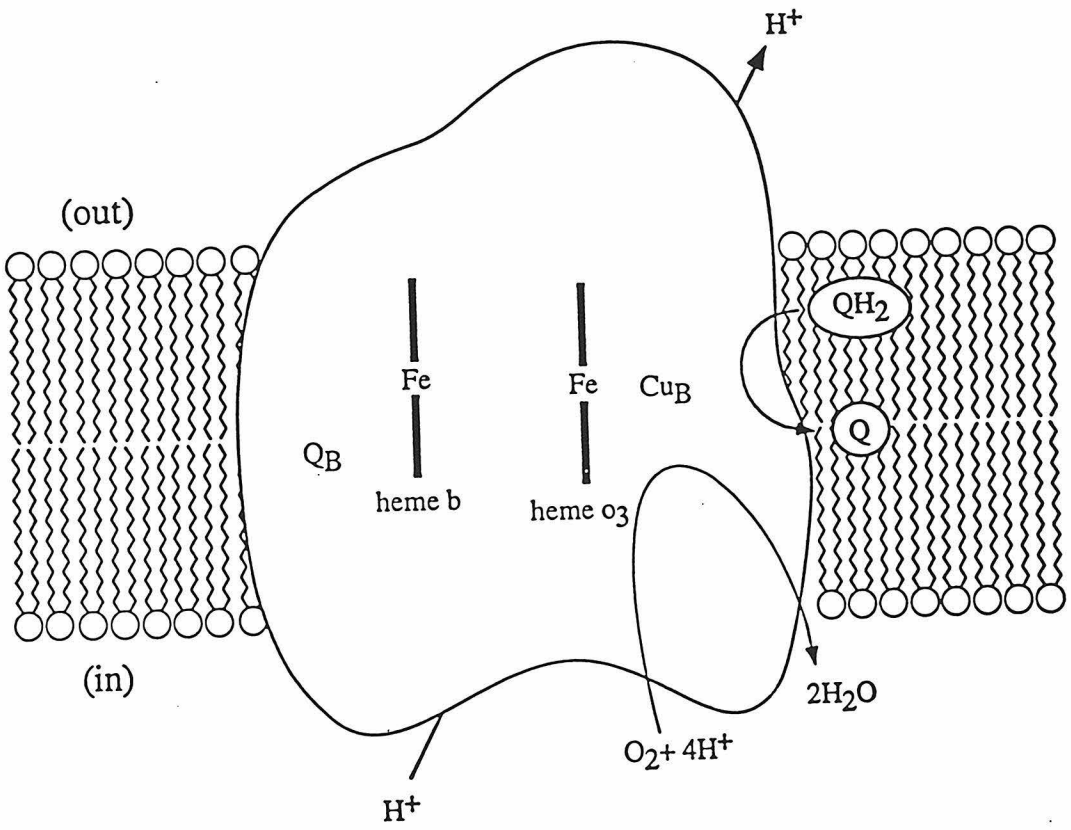


Figure 1.4. ***Escherichia coli*** **Cytochrome bo_3 ubiquinol oxidase**

binuclear center and reduced to water. For every four electrons passed through the enzyme to dioxygen, eight protons are translocated across the cytoplasmic membrane[2]. The overall reaction of this enzyme is shown in equation:

$$2\text{Ubiquinol} + O_2 + 8H^+ (\text{in}) \rightarrow 2\text{Ubiquinone} + 2H_2O + 8H^+ (\text{out})$$

Due to the analogous oxygen chemistry and the similarity of subunit I structure, it has been proposed that the proton translocation mechanism of cytochrome bo_3 is the same as that of the cytochrome c oxidases, in which only the two hemes and Cu_B in subunit I are responsible for the proton translocation. In this hypothesis, the additional redox center in both enzymes (Cu_A in cytochrome c oxidase and Q_B in cytochrome bo_3) is assumed to function only as the electron transfer mediator. However, a different hypothesis proposed that the proton translocation of cytochrome bo_3 utilizes a Q-cycle mechanism analoguous to that of cytochrome bc_1[26]. This opinion was supported by the discovery that the Q_B quinone is exchangeable under turnover conditions and the affinities of sites Q_A and Q_B for quinols, semiquinones, and quinones are also in accord with Q-cycle mechanism[27].

The electron-transfer pathways described by the two mechanisms are radically different. In hypothesis one, an ion-pump model suggests that electron transfers in the order of ubiquinol $\rightarrow$ heme b $\rightarrow$ (heme O_3/Cu_B). Since ubiquinol is a two-electron donor whereas heme b is a one-electron redox center, this model requires either the transient formation of the Q_A semiquinone or a split electron-transfer pathway in which one electron is passed to heme b and the second one is transferred elsewhere[31]. Sato-Watanabe et al. have proposed that the quinone in the Q_B site accepts the second electron and the electrons could be transferred sequentially through heme b to the binuclear site. The release of four protons from the oxidation of two quinol molecules, along with the pumping of four protons during oxygen reduction, would account for the observed proton translocation stoichiometry.

In contrast, the Q-cycle hypothesis for cytochrome bo_3 postulates a split electron-transfer pathway in the oxidation of quinol, which is analogous with that in the cytochrome bc_1 complex. In this model, for the two electrons from the quinol oxidation, one electron travels to the high-potential Cu_B site and the other electron passes through heme b to reduce Q_B to semiquinone. In the oxidation of a second quinol, Q_B is reduced to a quinol and is then replaced by another molecule of quinone. The oxidation of four quinol molecules releases eight protons on the outside of the plasma membrane, and the reduction of two quinone molecules, coupled with the uptake of four protons from the inside of the membrane, would account for the observed proton translocation stoichiometry. In this model, the observed $8H^+/4e^-$ proton-pumping ratio arises solely from quinol oxidation; no ion pumping occurs, and the binuclear heme-copper site only serves as an electron sink.

Thesis Focus

In light of the above discussions, there is a need for the accurate determination of the kinetics of enzymatic turnover events. To understand the detailed enzymatic mechanism of the ubiquinol oxidizing enzymes, it is critical to measure the individual kinetic steps and the thermodynamics of each event involved in enzymatic turnover. In the past fifteen years, methods for photoinduced one electron input into proteins have been developed to study electron transfer in proteins. For example, by examining the fast kinetic events in cytochrome c oxidase using the rapid photoreduction of cytochrome c, a detailed understanding of the mechanism of catalysis was obtained[19]. While such

methods are well suited for single electron donations, they are not suited for the required two electron reduction of the ubiquinol oxidizing enzymes. A novel approach is needed to generate a two-electron donating species rapidly and readily, surpassing the time resolution capabilities of current rapid mixing techniques.

The goal of this research is to design a technique that can rapidly trigger on the electron transfer reactions in ubiquinol oxidizing enzymes so that rapid electron transfer events under single turnover conditions can be studied in a time-resolved fashion. To achieve this goal, the strategy of inactivating quinol substrate with a photolabile protecting group will be pursued. Ideally, the ubiquinol would be released in submillisecond time scale upon photoflash so that the kinetics for each individual electron transfer steps in enzymes can be discerned.

Since structural factors have been suggested to play a key role in electron-transfer gating mechanisms, a photoreleasable ubiquinol might also be used in time-resolved crystallographic study for obtaining direct dynamics information on the protein conformation changes during electron transfer.

In this report, chapter 2 will describe the design of caged ubiquinol in more details. Chapter 3 and 4 will describe the synthesis and photolysis properties of 3',5'-dialkoxybenzoin caged ubiquinols. Chapter 5 will describe the synthesis of ubiquinol analogues attached with a carboxylic acid group. These analogues can be caged via an ester bond, which will lead to faster release than the caged ubiquinols described in chapter 3 and 4. In this chapter, the design and synthesis of mercaptopyridine N-oxide caged ubiquinol will also be briefly described. Finally chapter 6 and 7 will describe the

synthetic study and quantum chemistry simulation studies on the photolysis mechanisms of benzoin esters.

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Chapter 2

Design for the Rapid Triggering of the Two-electron Donor Release

Methods for the Rapid Triggering of Electron Transfer

The study of rapid electron-transfer events in redox active enzymes is critical for understanding the detailed enzymatic mechanism of these proteins. However, current stopped flow methods are limited in this respect since the mixing dead time is typically a millisecond or more. Although sub-millisecond rapid mixing has been achieved recently[25], it is often very inappropriate for biological studies since the highly turbulent flow makes it difficult to maintain structural integrity of the biological samples. The photochemical initiation of electron transfer has been a more appropriate approach in triggering biological electron transfers[8]. In biological systems, there has been a long history of the study of light-induced electron transfer using the photochemical excitation of chlorophyll or bacteriochlorophyll to initiate electron transfer processes. However, new techniques have been developed in the past decade to allow investigators to probe electron transfer in a wide variety of systems using light to initiate reactions. Among those, laser flash photolysis technique is of particular interest, since it allows rapid initiation of electron transfer (ns time domain) as opposed to the 1-2 ms limitation of mixing methods. Among the proteins studied to date, most are c-type cytochromes, iron-sulfur proteins, b-type cytochromes, copper containing protein and flavoproteins. In particular, redox proteins containing multiple redox centers are amenable to investigation using photochemical initiation. An example is the study on 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[14, 11, 29, 5, 18, 19, 20].

While such methods are well suited for single electron donating or accepting proteins such as cytochrome c, redox active enzymes such as cytochrome bc_1 and cytochrome bo_3 are ubiquinol oxidizing enzymes and require a rapid two electron reduction of ubiquinone to form ubiquinol in order to initiate the reaction. A novel approach reported to resolve 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[22]. Unfortunately, the rate for ubiquinol to release from the PRC is still slow in comparison with typical ubiquinol oxidation rates for enzymes such as the cytochrome bc_1, bo_3 or b_6f complex. Consequently, a more rapid method is required for releasing ubiquinol on a sufficiently fast time-scale to allow for kinetic resolution.

Toward this end, a "caged ubiquinol" strategy was designed and employed in the current investigation.

Design of Caged Ubiquinols

Caged Compounds

"Caged compounds" are synthetic molecules in which a biologically active molecule is attached with a photolabile protecting group. Consequently, its biological activity is controlled by light, i.e., by photolytic conversion from an inactive to an active form. In applications, covalent bond formation is usually employed to mask some feature important for biological recognition. Photochemical cleavage of the covalent bond will release the active species. Since illumination can be easily controlled in timing, location and amplitude, abrupt or localized changes in concentration of active species can be

achieved with controlled amplitudes. This capability is particularly valuable when rapid mechanical mixing is impractical, for example, when kinetic study in an intact cell, tissue or protein crystal is desired. One of the most exciting applications that have been established is the one-cycle activation of enzyme substrates. Rapid photocleaveage with a concomitant rapid change in substrate concentration is achieved with a fast pulse of near-UV light. This technique has made it possible to study sub-millisecond process that was not accessible by stop-flow methods due to the dead time of mixing and diffusion artifacts. Applications of this method range from the use of caged ATP to study its effects on muscle physiology and active cell transport, to the use of photosensitive ion chelators in the study of transmitter release and membrane transport. Photolysis of caged compounds has been one of the widely used techniques to examine the fast kinetics or spatial heterogeneity of biochemical responses in cell biology and biochemistry[1, 7].

Caged ubiquinol is designed by modifying the ubiquinol molecule or its analogues with a suitable photoremovable protecting group or caging group. In order to achieve sub-millisecond or even faster release of biologically active ubiquinol, it is very critical to choose the appropriate caging group. To be used in this research, this caging group must meet several criteria. First, it should render the caged ubiquinol or its analogues inactive as a two-electron donor to the enzymes studied. Second, it should release the ubiquinol or its analogues in high yield by photolysis at wavelengths of light that are non-detrimental to the protein samples. The quantum yield of photolysis must be high enough to produce readily measurable catalytic events for kinetic studies. Third, to ensure kinetic resolution, the photolysis rate should exceed the enzymatic turnover rate

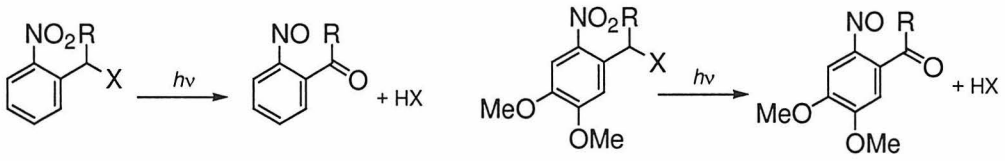
by several orders of magnitude. Since the turnover rates for both cytochrome bc_1 and cytochrome bo_3 are faster than millisecond time domain, preferably, ubiquinol should be released in microsecond time scale or even faster. In addition, any photoreaction by-products other than the desired ubiquinol or its analogues should be inert.

Caging Groups

Several different caging groups have been described and used in biological applications. In order to select the most appropriate caging group for our investigation, major classes of the available caging groups are examined:

2-Nitrobenzyl Groups

Caging groups based on the photoisomerization of 2-nitrobenzyl



2-nitrobenzyl (NB) (R = H)
2-(2-nitrophenyl)ethyl (NPE) (R = Me)

4,5-dimethoxy-2-nitrobenzyl (DMNB) (R = H)
2-(4,5-dimethoxynitrophenyl)ethyl (DMNPE) (R = Me)

Figure 2.1. 2-Nitrobenzyl Caging Groups

susbstituents are by far the most prevalent in present caged compounds. The synthesis and photocleavage properties of a series of substituted nitrobenzyl protecting groups have been summarized by Woodward[21]. Their advantages include the ability to cage a wide

variety of functionalities (e.g., phosphates, carboxylates, hydroxyl groups, amines and amides), ease of synthesis and reasonable light sensitivity and kinetics[15]. Subsequently, a variety of nitrobenzyl protecting groups have played important roles in synthetic chemistry as well as being used in such diverse fields, such as semiconductor lithography[24] and the study of rapid enzymatic processes[10,15].

A major problem of the 2-nitrobenzyl (NB) group is that the photoproduct, 2-nitrosobenzaldehyde, is reactive with many other components[12]. As an improvement, 1-(2-nitrophenyl)ethyl (NPE) group (Figure 2.1) generates the less-reactive acetophenone and is less toxic, particularly in the presence of scavengers such as thiols and semicarbazide[12]. However, it is not completely inert before photolysis and the substrate releasc rate is modest. The 4,5-dimethoxy-2-nitrobenzyl (DMNB) cage (Figure 2.1), although the kinetics of release is much faster than for the corresponding NPE caging group, has a low quantum yield[31]. The 1-(4,5-dimethoxy-2-nitrophenyl) ethyl (DMNPE) cage, which combines both modifications in the NPE and DMNB groups, has also been investigated. However, the release kinetics with ATP[31] and amino acids was found to be rather slow[30]. Although considerable biological success has been achieved in the use of NPE and DMNB caged compounds, especially with phosphate ester and anhydrides, the speed of release of the substrate and the ability to photolyze efficiently at longer wavelengths still can not match the requirement for our application.

3-Nitrophenyl ester and its derivatives

Another class of reported caging groups is 3-nitrophenyl ester and its derivatives, such as 3,5-dinitrophenyl (Figure 2.2), which undergo photosolvolysis to release the phosphate and the nitrophenol[13]. 3,5-nitrophenyl (DNP) phosphate has been used to photorelease phosphate in crystals of glycogen phosphorylase b to allow monitoring of the catalytic cycle by Laue X-ray diffraction[9]. The DNP-caged phosphate is photolyzed by irradiation at 300-360 nm with reasonable quantum efficiency (0.67) and release phosphate at $>10^4$ s^{-1} [6]. One problem with this protecting group is the danger of other nucleophiles (such as lysine side chains) competing in the photosolvolysis and leading to protein modification. In addition, the photolysis rate of this caging group is not fast enough for our research.

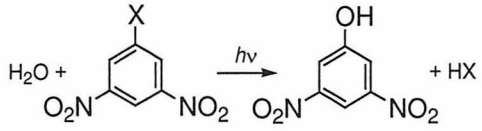


Figure 2.2. 3,5-Dinitrophenyl Caging Group

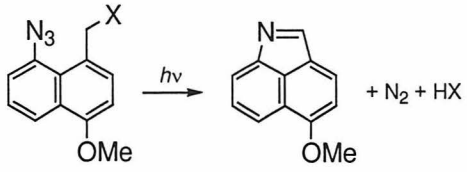


Figure 2.3. (4-Methoxyl-8-azido-1-naphthyl)methyl Caging Group

(Azidonaphthyl)methyl esters

Another type of protecting groups used for photogeneration of carboxylates is (azidonaphthyl)methyl esters. Their photolysis generates a nitrene that inserts into peri-methylene group and triggers elimination of the molecule being caged[4]. One problem in applying this group as a protecting group is the danger of nitrene insertion at other sites, which would result in potential biomolecule or protein modifications.

5,7-Dintroindolinylamide and methoxyphenacyl groups

These two types of caging groups are listed (Figure 2.4) because both of them have been observed to possess favorable photolysis properties for caging applications. However, no detailed photochemical characterizations for them, such as quantum efficiency, absorbance coefficient, or kinetics of release have been reported.

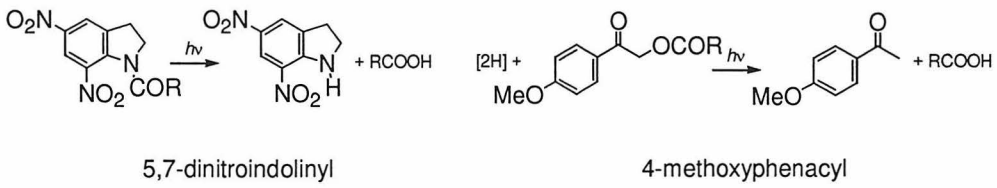


Figure 2.4. Dinitroindolinyl and Methoxyphenacyl Caging Groups

5,7-Dintroindolinylamide can be irradiated at wavelength beyond 400 nm and is much more photosensitive than the DMNB group for the release of acetates[2]. Methoxyphenacyl groups can be photolyzed by irradition of greater than 330nm[26].

Methoxyphenacyl demonstrated better photolysis properties than the NPE group for blocking inorganic phosphate.

N-hydroxy-2-thiopyridone group

N-hydroxy-2-thiopyridone derivatives have been reported to produce a variety of oxygen centered radicals by N-O bond cleavage[28, 3]. The photolysis of the aliphatic or alicyclic derivatives of thiohydroxamic acids is a convenient source of carbon radical. Newcomb and his colleagues have shown that the corresponding carbamate derivatives are the progenitors for nitrogen radicals[16]. The analoguous carbonate derivatives of N-hydroxypyridine-2-thione carbonates have also been found to generate alkoxycarbonyloxy radicals.

In the photolysis of N-hydroxy-2-thiopyridone derivatives, HTOC-NR_2, HTOC-R and HTOC-OR, cleavage of the N-O bond to give carbamoyloxy, acyloxy or alkoxycarbonyloxy radicals, respectively (Scheme 5.1). Rapid decarboxylation ($> 10^8\ s^{-1}$) occurs when the radical thus formed is stable. However, when the incipient radical is unstable (e.g., R in HTOC-R is aryl, vinyl, etc.), the decarboxylation step is slow and the radicals may decompose via other reaction channels[17] (Figure 2.5).

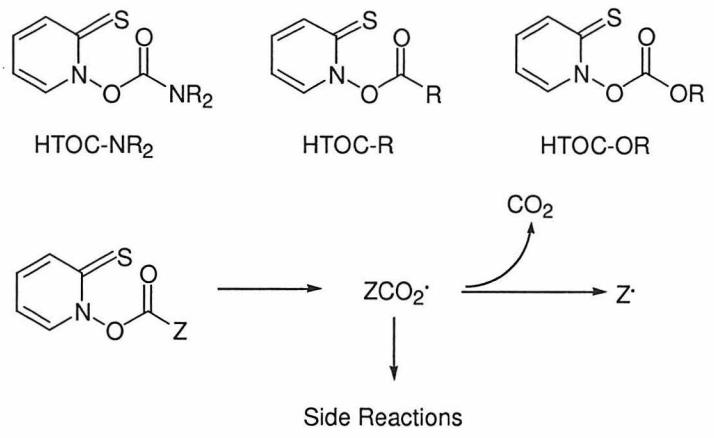


Figure 2.5. Photolysis of N-hydroxy-2-thiopyridone Derivatives

3', 5'-Dimethoxybenzoin esters

Substituted benzoin compounds were introduced by Sheehan[27] in 70s as potential photosensitive protecting groups for carboxylates. Among these compounds, 3',5'-dimethoxybenzoin is reported to possess most remarkable photolysis properties. Its esters have been reported to photolyze with rates exceeding 10^9 s^{-1} at quantum yield of 0.78[23]. The photolysis by-product is non-reactive 5,7-dimethoxy-2-phenyl-benzofuran. Due to these desirable photochemical properties, there have been recurring interests in 3', 5'-dimethoxybenzoin in recent years. DMB has been used to cage a wide variety of biologically interesting molecules and different functional groups, such as carboxylic acid, hydroxyl and amine.

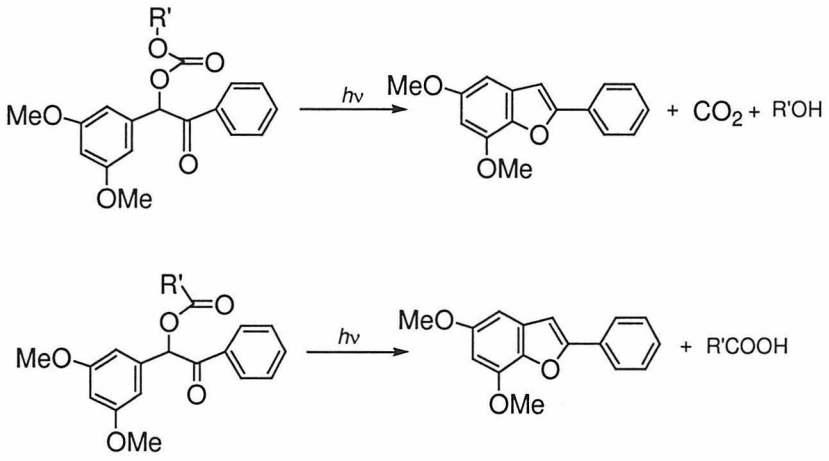


Figure 2.6. Photolysis of 3',5'-Dimethoxybenzoin Esters

Proposed Schemes for Caged Ubiquinol

In our investigation of the rapid electron transfer in ubiquinol oxidizing protein complexes, we decided to employ substituted benzoin, starting with 3',5'-dimethoxybenzoin as the caging group to cage ubiquinol and its biologically active analogues. Oxycarbonyl (OCO) linkage will be used to link to a hydroxyl group in ubiquinol to inactivate the two-electron donor. Upon photolysis, oxycarbonyl linkage will be broken to release CO_2, DMB will be converted to benzofuran and free ubiquinol will be released.

Ubiquinol caged with N-hydroxy-2-thiopyridone will also be investigated. By photolysis in the presence of an efficient hydrogen-donating reagent, it is expected that a rapid photorelease of ubiquinol can be achieved via a rapid hydrogen uptake by semiquinone radical formed upon photolysis.

Another caging strategy is to cage a biologically active ubiquinol analogue attached with a carboxylic acid group via an ester bond. As described in chapter 5, a number of such ubiqinonol analogues are synthesized and will be screened for their bioactivity.

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Chapter 3

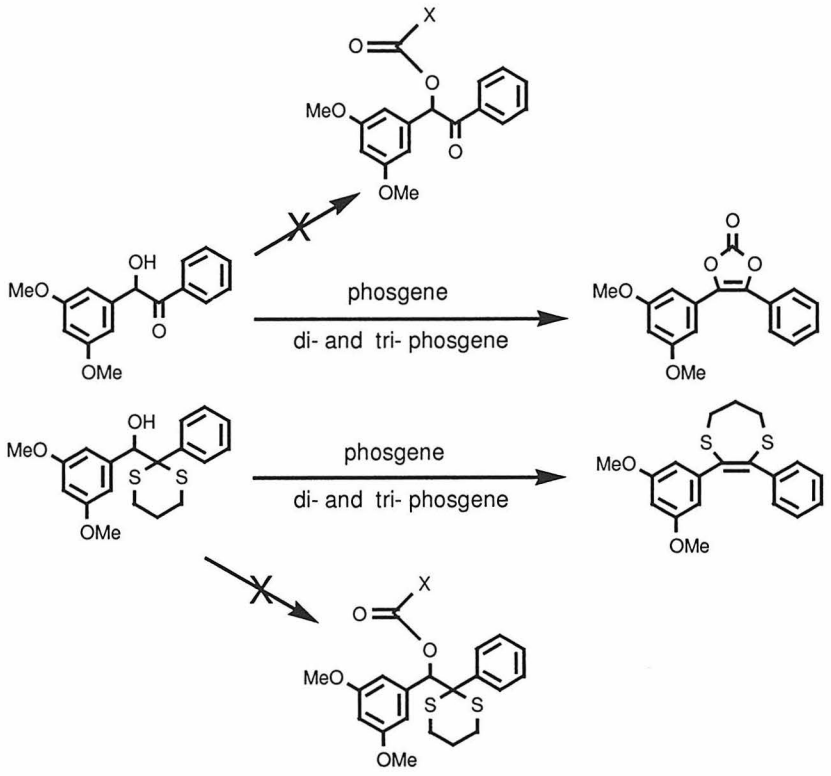
3′, 5′-Dimethoxybenzoin Caged Ubiquinol

Result and Discussion

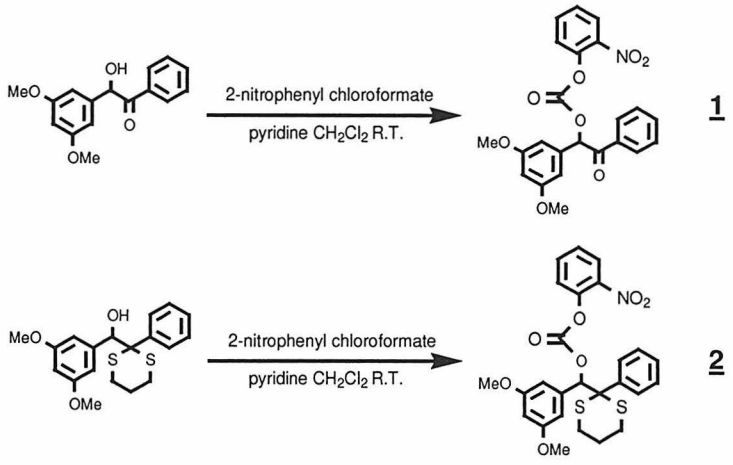
Formation of the Photolabile Carbonate Linkage

A critical step in the synthesis of DMB caged ubiquinol is the formation of the carbonate linkage between ubiquinol and DMB. Several methods have been investigated for the formation of an activated carbonate DMB. These included the use of phosgene, di- and tri-phosgene. These methods were tested and were found to be inefficient for our applications due to the formation of a cyclization product in nearly quantitative yield (Scheme 3.1). The dithiane protected DMB was also used in the ubiquinol coupling reactions with phosgene, di-and tri-phosgene and carbonyl diimidazole as well as the triflate salt of dimethyl carbonyl diimidazole. However, all of these reactions led to the formation of dehydration products in substantial (Scheme 3.1).

Efforts have been made to activate DMB or DMB dithiane by attaching them with 2-nitrophenol via carbonate linkage. The 2-nitrophenol carbonate activation was pursued because it has been reported[1] that such carbonates are highly reactive in the presence of DMAP and form a new carbonate linkage with another alcohol or phenol. At the beginning, the reagent, 2-nitrophenyl chloroformate, was used to activate DMB and DMB dithiane. Reactions of DMB and DMB dithiane with 2-nitrophenyl chloroformate in the presence of excess pyridine produced the desired mixed carbonate **1** and **2** in good yield.

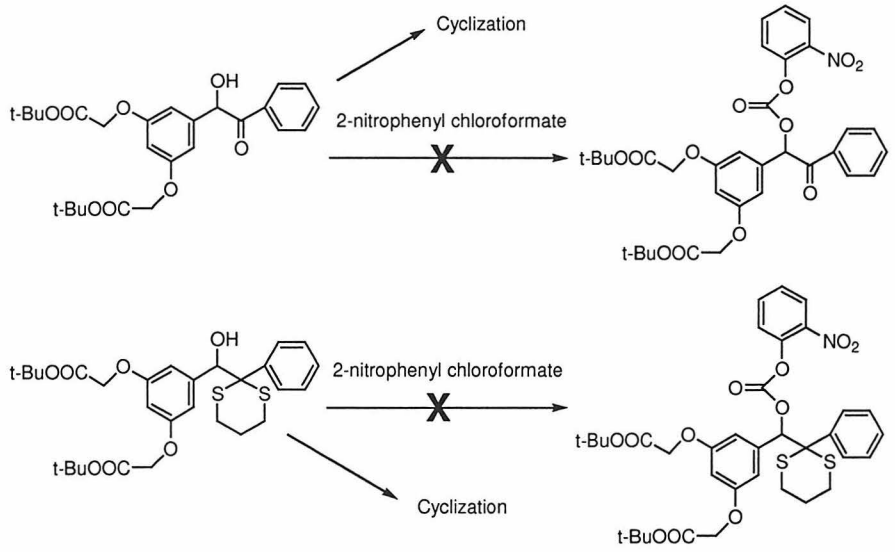


Scheme 3.1. Failed Reactions in Carbonate Linkage Formation

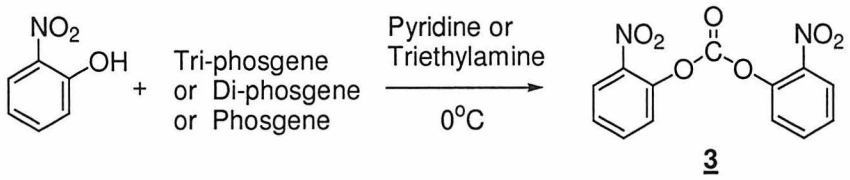


Scheme 3.2. Cage Activation by 2-Nitrophenyl Chloroformate

However, when 2-nitrophenyl chloroformate was used afterwards in the reactions with analogues of DMB and DMB dithiane, dehydration products were obtained in nearly quantitative yield again (Scheme 3.3). It was suspected that the 2-nitrophenyl chloroformate purchased from commercial sources might contain phosgene since it is normally prepared by a phosgene reaction. There are only two suppliers of 2-nitrophenyl chloroformate, Carbolabs Inc. and Aldrich Inc. Several batches of 2-nitrophenyl chloroformate were ordered from both of the two suppliers and the same reaction was repeated a few times. The dehydration product was yielded every time. Therefore, a better synthetic approach to activate phenol or alcohol by 2-nitrophenol carbonate ester needs to be developed. Besides the inconsistent reaction results from using 2-nitrophenyl chloroformate, since 2-nitrophenyl chloroformate is a moisture-sensitive waxy solid at



Scheme 3.3. 2-Nitrophenyl Chloroformate Led to Cyclization Products



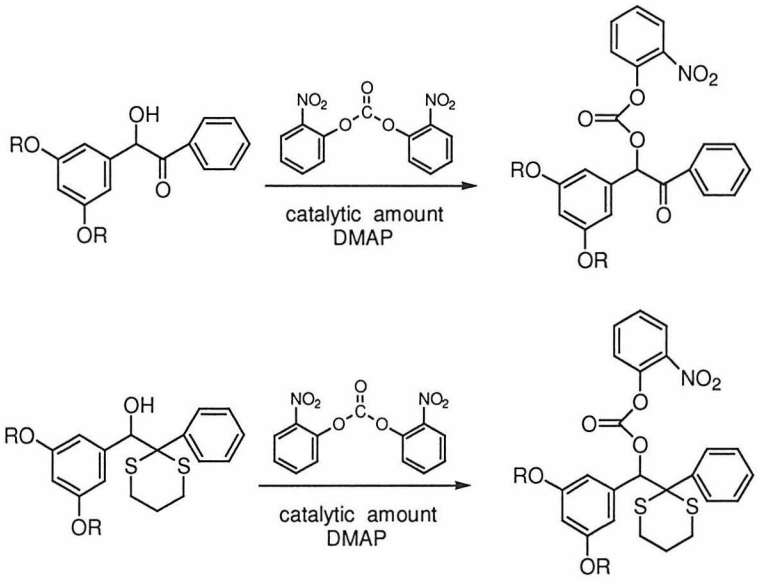
Scheme 3.4. Preparation of the Bis(2-nitrophenyl) Carbonate

room temperature, the experimental manipulation is also rather inconvenient. It is preferred that a new reagent could replace 2-nitrophenyl chloroformate in this activation reaction.

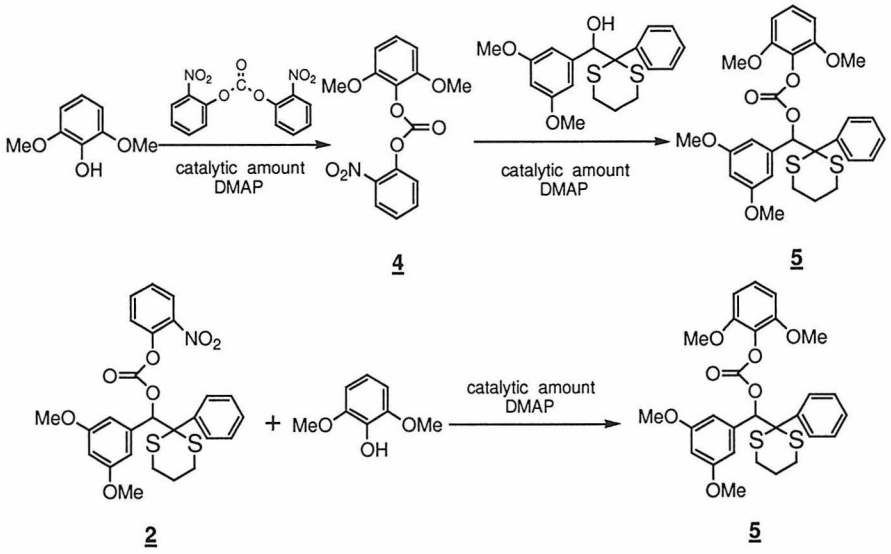
Towards this end, a new synthetic method using bis(2-nitrophenyl) carbonate was developed. Bis(2-nitrophenyl) carbonate is not commercially available; however, it can be easily prepared from 2-nitrophenol and phosgene (or diphosgene, triphosgene) in the presence of excess pyridine or triethylamine. The reaction mixture can be readily purified via regular work-up procedures and recrystallization. The obtained bis(2-nitrophenyl) carbonate is feather-like crystals and not moisture-sensitive; therefore its storage and manipulation are very convenient.

Under the catalysis of DMAP, Bis(2-nitrophenyl) carbonate reacts with DMB or the DMB analogues shown in Scheme 3.5 to afford the activated carbonate in a very good yield. The resultant activated DMB has been tested to react with alcohols, phenols, secondary and primary amines and thiols (Scheme 3.7). With catalytic amount of DMAP (DMAP is not needed in the reactions with amines), all the reactions led to the desired DMB caged compounds in good yields at room temperature.

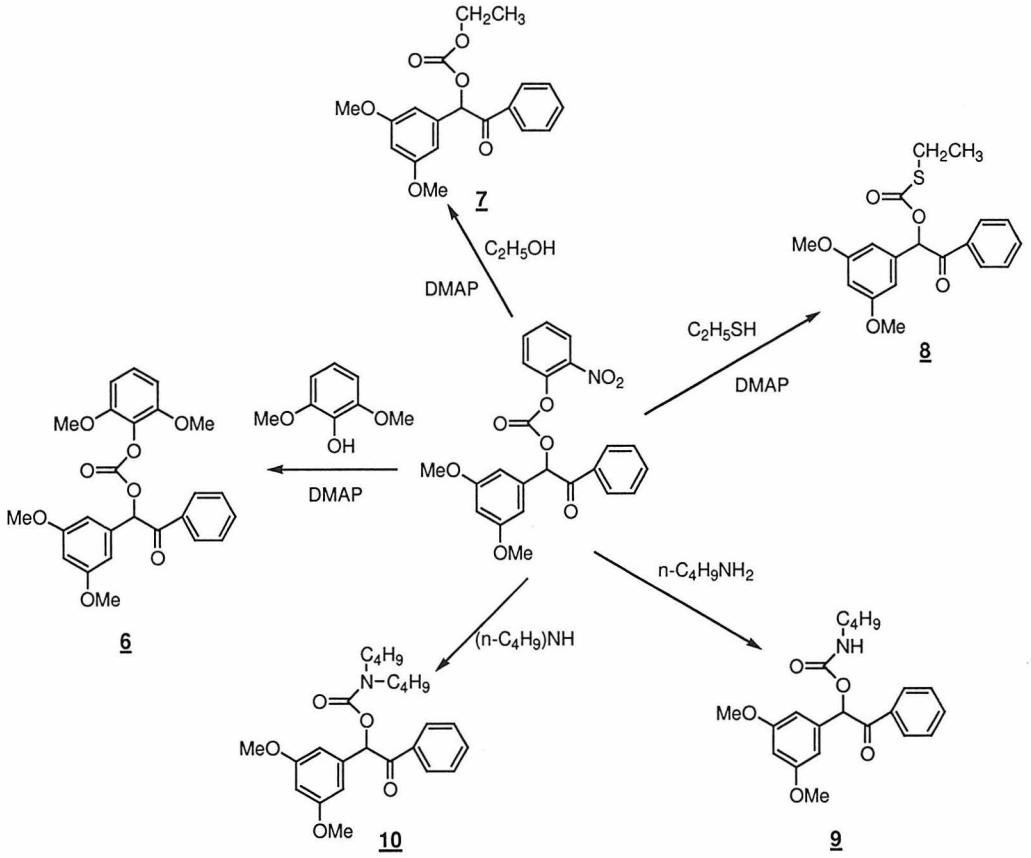
On the other hand, instead of activating cage compound, bis(2-nitrophenyl) carbonate can be used to first activate the substrate to be caged. The activated substrates react with the benzoin caging group to give the same final products in a similarly overall yield (Scheme 3.6). Such a synthetic scheme provides a general synthetic method for caging various substrates via carbonate linkage.



Scheme 3.5. Cage Activation by Bis(2-nitrophenyl) Carbonate



Scheme 3.6. Synthesis of the Caged Compound in Different Synthetic Procedures



Scheme 3.7. Reactions of Activated Cage with Alcohol, Phenol, Thio and Amines

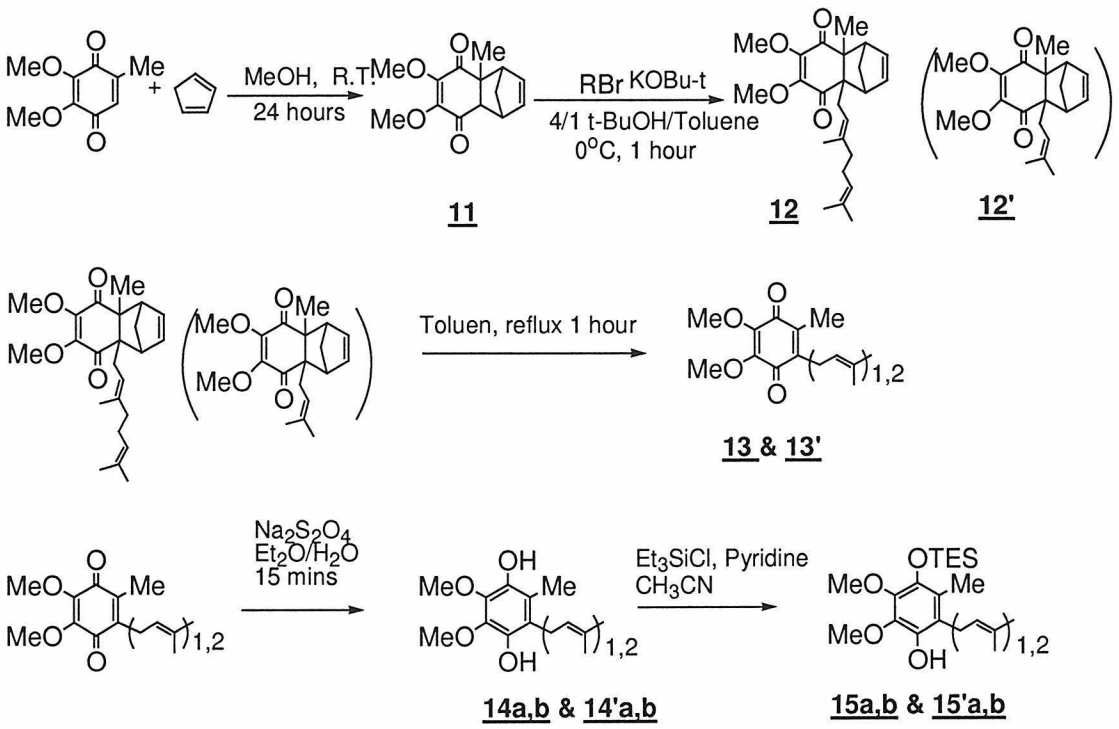
Synthesis of Ubiquinol

After the problem of carbonate linkage formation was successfully resolved, the next synthetic target would be the ubiquinone molecule. There have been a number of published methods on ubiquinone synthesis[2, 5, 6, 3]. The method described in reference 6 was pursued since the synthetic procedure is relatively simple and all the reagents used are at reasonable costs[4]. The synthesis utilized a Diels-Alder transformation of quinone to form the tricylodione (**11**). Alkylation and subsequent retro-Diels-Alder reaction of the alkylated tricyclodione (**12**) afforded the desired ubiquinone-1 and ubiquinone-2 in an overall yield of 73%.

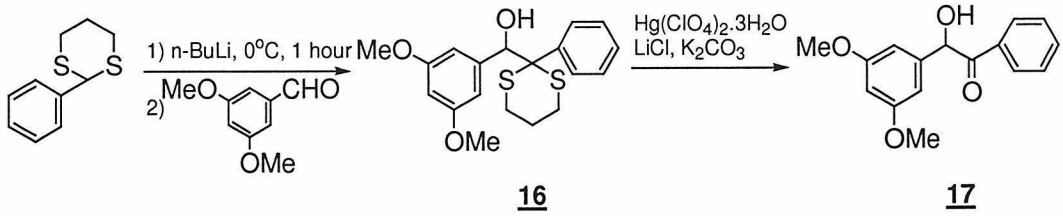
Since only one of the two hydroxyl groups need be caged, we chose to selectively silyate a single phenolic oxygen using triethylsilyl chloride. Treatment of ubiquinone with sodium dithioinite in aqueous methanol and extraction with hexane under nitrogen gave ubiquinol in quantitative yield. Silyation of the ubiquinol with triethylsilyl chloride in dry acetonitrile with pyridine afforded the monosilyl ubiquinol in satisfactory yields.

Synthesis of 3', 5'-Dimethoxy Benzoin

3',5'-dimethoxy benzoin was synthesized starting from 3,5-dimethoxy benzaldehyde and phenyl dithiane. The phenyl dithiane was treated with *n*-butyllithium at 0°C to form the dithiane anion. It was titrated by 3,5-dimethoxy benzaldehyde in THF solution. The dithiane DMB was obtained in nearly quantitative yield. The dithiane protecting group was removed by reacting with $Hg(ClO_4)_2$ to afford DMB in more than 90% yield.



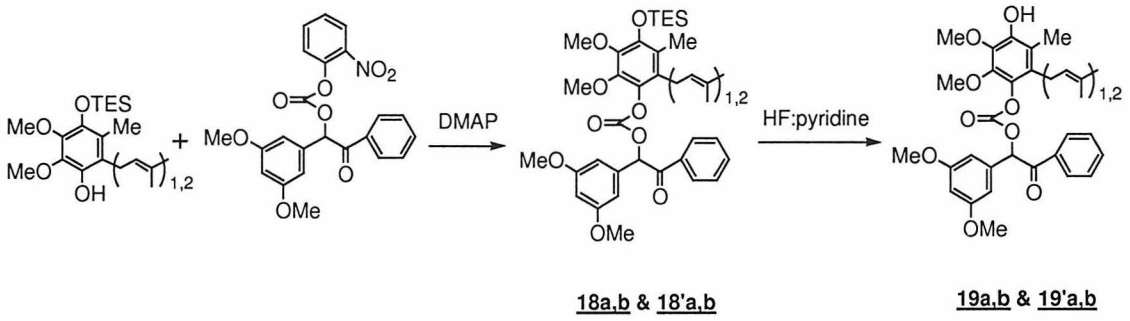
Scheme 3.8. Synthesis of Ubiquiol-1 and Ubiquinol-2



Scheme 3.9. Synthesis of 3',5'-Dimethoxybenzoin

Assembly of the Caged Ubiquinol (Scheme 3.10)

The monosilyl ubiquinol was subsequently coupled to the 2-nitrophenyl carbonate ester of 3', 5'-dimethoxybenzoin under the catalysis of DMAP in methylene chloride or acetonitrile. After purification, the triethylsilyl protecting group was removed by treatment with HF-pyridine in acetonitrile. The final product was purified by reverse phase HPLC to yield the desired "caged" ubiquinol as a clear colorless waxy solid.



18a,b & 18'a,b 19a,b & 19'a,b

Scheme 3.10. Assembly of the Caged Ubiquinol

Photolysis of the Assembled Caged Ubiquinol

Photochemical properties of the caged ubiquinol were characterized first to determine the usefulness of obtained compounds for rapid kinetic studies. The steady-state photolysis in the presence of 355nm light was demonstrated in Figure 3.1. Over the course of the photolysis, a clean transition from the starting material to products was observed. The increase in absorbance at 300 nm is due to the formation of benzofuran (BF). The isobestic point at 258 nm indicated that the photolysis occurs as a single transition from the starting material to products.

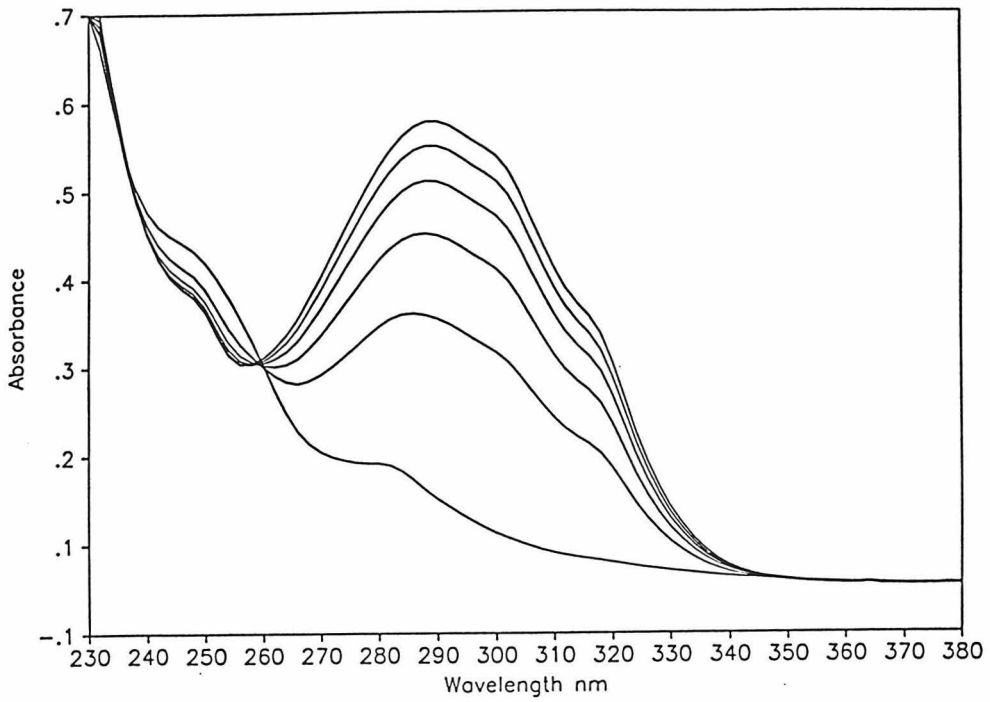


Figure 3.1. Photolysis of 3',5'-Dimethoxybenzion Caged Ubiquinol

Concomitant with the spectral changes observed is the formation of the desired ubiquinol and the expected benzofuran. Figure 3.2 shows a reverse phase HPLC trace of a number of standards as well as the caged ubiquinol-2 **19a, b**. The HPLC analysis indicated that ubiquinol-2, ubiquinone-2, and benzofuran are the sole products from photolysis. The presence of ubiquinone-2 was most likely due to small amount of oxidation that occurred during handling of the sample in the HPLC analysis. It should be noted that the extinction coefficient for uiquinol-2 is about 12 times 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 than 10:1 in the HPLC trace shown in Figure 3.2.

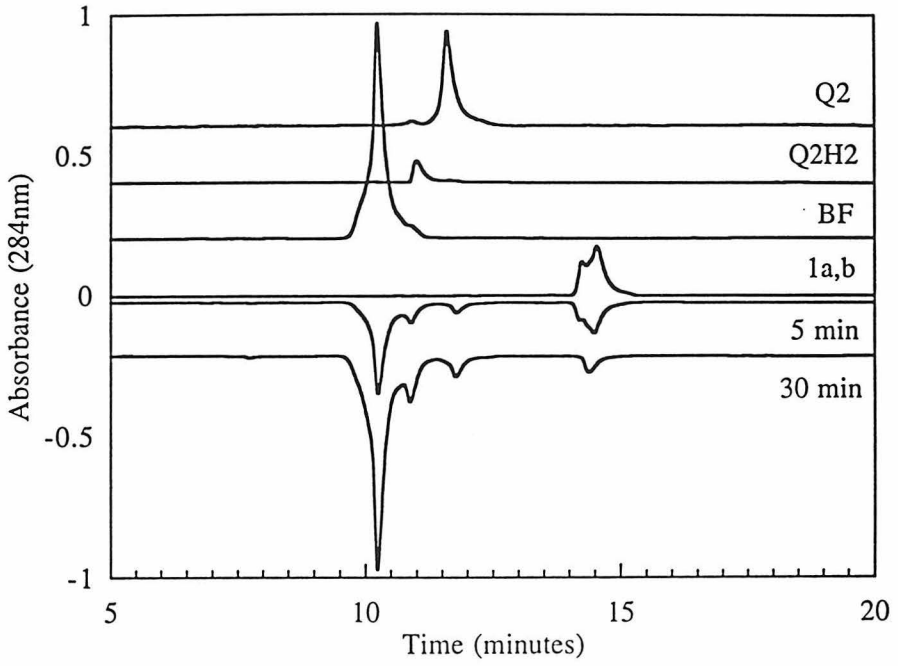


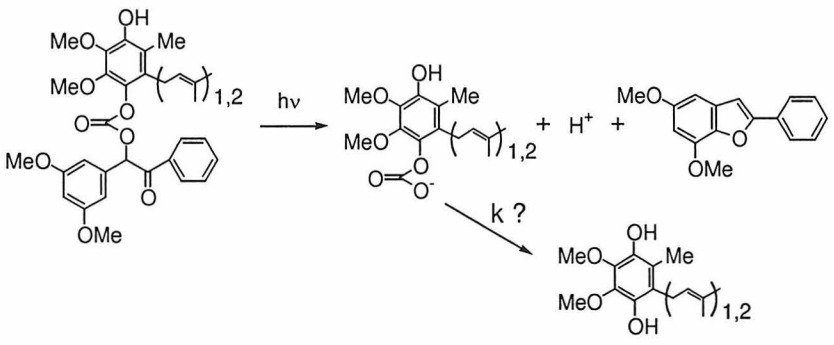
Figure 3.2. HPLC Analysis of the Photolysis Products

Transient absorption experiment was performed to determine the rate of benzofuran formation. The experiment indicated that the photoproduct is formed within the dead time of the instrument, placing a lower bound limit on the photolysis rate of 10^6 s^{-1}.

Apparently, the DMB caged ubiquinols possess very favorable photochemical properties for fast release of ubiquinols to trigger electron transfer in proteins. However, when these compounds were tested for water-solubility in aqueous solution, it was found that the water-solubility of DMB caged ubiquinols is very low and needs to be considerably improved for meaningful enzymatic experiments.

Summary

The synthesis of DMB caged ubiquinol-1 and ubiqunol-2 was achieved. Photolysis measurements of these compounds showed the rapid formation of benzofuran on the sub-microsecond time scale with concomitant release of ubiquinol monocarbonate ester (Scheme 3.11). However, it presented new problems that need to be resolved. First, the water-solubility of the caged ubiquinols needs to be improved for biological applications. Second, although the photorelease of ubiquinol monocarbonate ester is very rapid, in the time frame of sub-microsecond, the kinetics of free ubiquinol generation in aqueous solution (or in detergent buffer solution) from the ubiquinol monocarbonate ester needs to be characterized in detail. Ultimately, it is the formation rate of free ubiquinols that determines the feasibility of applying the caged ubiquinol for rapid electron transfer studies.



Scheme 3.11. Photolysis of DMB Caged Ubiquinol

On the other hand, from the viewpoint of synthetic chemistry, the methods described in this chapter are so general that a variety of compounds could be similarly caged.

Materials and Methods

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 purchased from Fluka. 2-Nitrophenyl chloroformate was purchased from Carbolabs, Inc., and Aldrich, Inc. NMR spectra were recorded on a GE QE300 spectrometer operating at nominal frequencies of 300 MHz. 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 12m silicon gum capillary column.

Synthesis of 2-nitrophenyl carbonate activated DMB with 2-nitrophenyl chloroformate (Scheme 3.2). A flame dried flask equipped with stirring bar and septum was charged with DMB or DMB dithiane (24.4mmol), 150mL of dry methylene chloride and 2-nitrophenyl chloroformate (25mmol) at room temperature under nitrogen. To this solution, 2.2 equivalents of dry pyridine was added dropwise. The solution was stirred for 1 hr at room temperature, and the solvent was removed *in vacuo*. The resultant residue was washed with dry ether, and the ether extract was filtered through a plug of anhydrous

$MgSO_4$. Solvent was removed *in vacuo* to yield thick oil, which produced a pale yellow solid when dried under high vacuum.

Compound **1**: Yield 71%. ^1H NMR ($CDCl_3$) δ 8.11 (dd, 1H), 7.94 (dd, 1H), 7.68 (m, 2H), 7.45 (m, 3H), 7.34 (m, 2H), 6.77 (s, 1H), 6.46 (t, 1H), 6.02 (d, 2H), 3.77 (s, 6H). HRMS (FAB), m/z (M^+) cacld. 437.3989 obsd. 437.3988.

Compound **2**: Yield 77%. ^1H NMR ($CDCl_3$) 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 bis(2-nitrophenyl) carbonate (Scheme 3.4). To a dichloromethane (250mL) solution of 2-nitrophenol (13.91g, 100mmol) and triphosgene (6.43g, 21.7mmol) cooled in an ice bath, triethylamine (14.03mL) was added dropwise under argon flow. After stirring for 4 hours, the mixture was poured into dilute hydrochloric acid solution and extracted with diethyl ether. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The resulting solid was crystallized from hexanes/ethyl acetate to yield 14.5g (94%) of white, feather-like crystals. ^1H NMR ($CDCl_3$) δ 8.191 (dd, 2H), 7.762 (t, 2H), 7.519 (m, 4H).

Synthesis of 2-nitrophenyl carbonate activated DMB with Bis(2-nitrophenyl) carbonate (Scheme 3.5). To a dichloromethane solution (50mL) of 3', 5'-dimethoxybenzoin (2.72g, 10mmol) and bis(2-nitrophenyl) carbonate (6.1g, 20mmol), DMAP (120mg, 1mmol) was added. After stirring for 4 hr, the solution was poured into dilute hydrochloric acid

solution and extracted with diethyl ether. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The resulting solid was separated by flash column chromatography (silica gel, 4:1 hexanes/ethyl acetate) to yield 4.24g (97%) of the product.

*Synthesis of **4** (Scheme 3.6)*: To a dichloromethane solution (50mL) of 2,6-dimethoxyphenol (0.154g, 1mmol) and bis(2-nitrophenyl) carbonate (0.61g, 2mmol), DMAP (12mg, 0.1mmol) was added. After stirring for 4 hr, the solution was poured into dilute hydrochloric acid solution and extracted with diethyl ether. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The residue was separated by flash column chromatography (silica gel, 4:1 hexanes/ethyl acetate) to yield 0.29g (91%) of the product. ^{1}H NMR ($CDCl_3$) δ 8.21 (m, 1H), 7.74 (m, 1H), 7.45 (m, 1H), 7.14-7.04 (m, 2H), 6.77 (d, 2H), 3.90 (s, 6H).

*Synthesis of **5** (Scheme 3.6)*: To a dichloromethane solution (50mL) of **4** (0.16g, 0.5mmol) and 3′, 5′-dimethoxybenzoin dithiane (0.36g, 1mmol), DMAP (12mg, 0.1mmol) was added. After stirring for 6 hr, the solution was poured into dilute hydrochloric acid solution and extracted with diethyl ether. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The residue was separated by flash column chromatography (silica gel, 4:1 hexanes/ethyl acetate) to yield 0.53 g (98%) of the product. ^{1}H NMR ($CDCl_3$) 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.

Synthesis of **5** with the activated DMB was performed as follows: To a dichloromethane solution (50mL) of 2,6-dimethoxyphenol (0.30g, 2mmol) and **2** (0.53g, 1mmol), DMAP (12mg, 0.1mmol) was added. After stirring for 6 hr, the solution was poured into dilute hydrochloric acid solution and extracted with diethyl ether. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The residue was separated by flash column chromatography (silica gel, 4:1 hexanes/ethyl acetate) to yield 0.54 g (99%) of the product.

*Syntheses of **6, 7, 8** (Scheme 3.7)*: Syntheses of **6, 7, 8** represented the reactions of activated DMB with phenol, alcohol and thio. Similar experimental procedures were followed: To a dichloromethane solution (50mL) of substrate (5mmol) and **1** (0.36g, 1mmol), DMAP (12mg, 0.1mmol) was added. TLC was used to monitor the reactions. After compound **1** disappeared, the solution was poured into dilute hydrochloric acid solution and extracted with diethyl ether. The organic extract was dried over anhydrous magnesium sulfate and evaporated. The residue was separated by flash column chromatography (silica gel, 4:1 hexanes/ethyl acetate) to yield the product.

Compound **6**: ^{1}H NMR ($CDCl_3$) δ 7.70 (m, 2H), 7.39 (m, 1H), 7.29 (m, 2H), 7.12 (t, 1H), 6.59 (d, 2H), 6.35(t, 1H), 6.05 (d, 2H), 5.9 (s, 1H), 3.75 (s, 6H), 3.59 (s, 6H). HRMS (FAB) m/z (M^+) calcd. 452.4533 obsd. 452.4532.

Compound **7**: ^{1}H NMR ($CDCl_3$) δ 7.94 (m, 2H), 7.50 (m, 1H), 7.40 (m, 2H), 6.85(s, 1H), 6.60 (d, 2H), 6.41 (t, 1H), 4.25 (q, 2H), 3.74 (s, 6H), 1.32 (t, 3H). HRMS (FAB) m/z (M^+) calcd. 344.3585 obsd. 344.3585.

Compound **8**: ^{1}H NMR ($CDCl_3$) δ 7.80 (m, 2H), 7.47 (m, 1H), 7.39 (m, 2H), 6.45(t, 1H), 6.18 (d, 2H), 5.82 (s, 1H), 3.43 (q, 2H), 3.59 (s, 6H), 0.95 (t, 3H). HRMS (FAB) m/z (M^+) calcd. 360.4251 obsd. 360.4251.

*Syntheses of **9, 10** (Scheme 3.7)*: Syntheses of **9, 10** represented the reactions of activated DMB with primary and secondary amines. In contrast to the reactions with alcohol and thio, reactions with amine do not require the addition of DMAP. Product formation and the relcase of 2-nitrophenol were completed within a few minutes upon the addition of amines. A typical experimental procedure: to a dichloromethane solution (50mL) of **1** (0.36g, 1mmol), the amine (1mmol) was added. TLC was used to monitor the reactions. Normally after 2 - 10 minutes, the solvent was removed under reduced pressure and the residue was separated by flash column chromatography (silica gel, 4:1 or 2:1 hexanes/ethyl acetate) to yield the product.

Compound **9**: ^{1}H NMR ($CDCl_3$) δ 7.93 (dd, 2H), 7.54 (m, 1H), 7.41 (m, 2H), 6.47 (d, 2H), 6.35 (t, 1H), 5.85 (s, 1H), 3.78 (s, 6H), 2.91 (t, 2H), 1.90-1.70 (m, 4H), 1.03 (t, 3H). MS (FAB) m/z (MH^+) calcd. 372 obsd. 372; m/z (MNa^+) calcd. 394 obsd. 394

Compound **10**: ^{1}H NMR ($CDCl_3$) δ 7.92 (dd, 2H), 7.52 (m, 1H), 7.40 (m, 2H), 6.44 (d, 2H), 6.30 (t, 1H), 5.95 (s, 1H), 3.77 (s, 6H), 3.19 (t, 4H), 1.42 (m, 4H), 1.22 (m, 4H),

0.91 (t, 6H). MS (FAB) m/z (MH^+) calcd. 428 obsd. 428; m/z (MNa^+) calcd. 450 obsd. 450.

*Synthesis of **11***. A 25mL flask was charged with a stir bar and 5mL of dry methanol. This solution was added 3mL of freshly distilled cyclopentadiene. The reaction was 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 yield a pale yellow oil. Yield 0.75 g, (>99%). ^{1}H NMR ($CDCl_3$) δ 6.15 (m, 1H), 6.00 (m, 1H), 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 **12***. A flame dried flask was charged with geranyl bromide (1.44 g, 6.6mmol), **11** (1.5g, 6mmol), a stir bar, and 40mL of dry 1:3 toluene/*tert*-butyl alcohol. This solution was cooled to 0°C using an ice bath. 10mL of 1.0M potassium *tert*-butoxide in *tert*-butyl alcohol was added via cannula with rapid stirring. The solution immediately turned to a dark brown color. The reaction was allowed to stir for 1 h at 0°C. The mixture was then poured into 50mL of saturated NH_4Cl and extracted with 2 x 100mL of diethyl ether. The organic layers were combined, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The resultant dark brown oil was purified by flash column chromatography using 4:1 hexane/ethyl acetate to yield 1.68 g (73%) of **12**. ^{1}H NMR ($CDCl_3$) 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 (d, 3H), 1.57 (s, 3H), 1.58 (s, 3H), 1.49 (s, 3H), 1.78 (d, 1H); HRMS (FAB) (MH^+) m/z calcd. 384. 230060, obsd. 384. 228649.

For **12'**: ^1H NMR ($CDCl_3$) δ 6.04 (bs, 2H), 5.07 (m, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.08-3.00 (d, 2H), 2.80-2.30 (dd, 2H), 1.76-1.42 (dd, 1H), 1.49 (s, 3H), 1.46 (s, 3H), 1.43 (s, 3H); HRMS (FAB) (MH^+) m/z calcd. 316.3915, obsd. 316. 3915.

*Synthesis of **13***. A stirred solution of **12** (1.68 g) dissolved in 20 ml of toluene was refluxed for 60 min. During this time the solution turned from pale yellow to reddish orange. Solvent was removed *in vacuo* and the resultant oil was purified by flash column chromatography using 4:1 hexane/ethyl acetate. Yield 1.4 g (>99%). ^1H NMR ($CDCl_3$) δ 5.03 (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.

For **13'**: ^1H NMR ($CDCl_3$) δ 4.93 (t, 1H), 3.99 (s, 3H), 3.97 (s, 3H), 3.18-3.16 (d, 2H), 2.01(s, 3H), 1.74 (s, 3H), 1.67 (s, 3H). HRMS (FAB) m/z (MH^+) calcd. 250.2903, obsd. 250.2903.

*Synthesis of **15a,b***. A round-bottom flask equipped with stir bar was charged with **13** (0.63 g, 2.0mmol) and 10mL of 9/1 methanol/water. With vigorous stirring, sodium dithionite (0.38 g, 2.2mmol) was added. The reddish orange color of the ubiquinone-2 quickly dissipated. 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). Chlorotriethysilane (0.33 g, 2.2 mmol) was added dropwise using a syringe pump over a 6 hr period. The reaction was monitored by GC/MS. Solvent and excess TESCI 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 for **15a** ($CDCl_3$) 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 for **15b** ($CDCl_3$) 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 **15a** and **15b**.

For **15'a,b** isomers: ^{1}H NMR ($CDCl_3$) δ 5.36 (s, 1H), 4.95 (m, 2H), 3.81 (s, 3H), 3.75 (s, 3H), 3.29 (d, 2H), 2.09 (s, 3H), 1.72 (s, 2H), 1.66 (s, 2H), 1.56(s, 6H), 0.95 (t, 9H), 0.73 (q, 6H). HRMS (FAB) m/z (MH^+) calcd.366.5671, obsd. 366.5672.

*Synthesis of **16**.* A flame dried flask was charged with a stir bar, 100mL of dry THF and 2-phenyl-1,3-dithiane (3.9 g, 20 mmol). This solution was cooled to 0°C and 22mmol of *n*-butyllithium was added dropwise via syringe (11mL of 2.0M solution in hexane). Formation of the dithiane anion was indicated by the appearance of a pale olive green color. Upon completion of the addition, the reaction was stirred for 30 minutes. Then 3, 5-dimethoxy benzaldehyde dissolved in dry THF was added via syringe (3.3 g, 20mmol,

2 x 10mL THF). The reaction was allowed to stir for 1 hr at 0°C and then poured into 100mL of 0.1M HCl and extracted with diethyl ether. The organic phase was dried over anhydrous magnesium sulfate, and the solvent was removed *in vacuo* to yield a colorless oil which was further purified by flash chromatography using 4/1 hexane/diethyl acetate. Yield: 7.1 g (98.6%). ^{1}H NMR ($CDCl_3$) δ 7.73 (dd, 2H), 7.31 (m, 3H), 6.30 (t, 1 H), 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 **17**.* To a mixture of **16** (3.6g, 10mmol), lithium chloride (0.4g 10mmol), water (3mL) in 30mL THF solution, Mercury perchlorate trihydrate (9g, 20mmol) was added. The mixture was stirred for 15 minutes and K_2CO_3 solution was added to adjust the pH to 7-9. The mixture was extracted with diethyl ether and the organic extract was dried over anhydrous magnesium sulfate, evaporated under reduced pressure. The residue was separated by flash column chromatography using 2:1 hexanes/ethylacetate to yield 2.56g (94%) of **17**. ^{1}H NMR ($CDCl_3$) δ 7.93 (dd, 2H), 7.54 (m, 1H), 7.41 (m, 2H), 6.47 (d, 2 H), 6.35 (t, 1H), 5.85 (s, 1H), 3.78 (s, 6H). HRMS (FAB) m/z (MH^+) calcd. 272.2958, obsd. 272.2958.

*Synthesis of **19a,b**.* A flame dried round-bottom flask was charged with **15a,b** (0.44g, 1mmol), **1** (0.44g, 1mmol) and 25mL anhydrous acetonitrile. DAMP (1mmol) was added and the mixture was stirred for 3 days and poured into saturated ammonium chloride solution. The mixture was extracted with diethyl ether and the organic phase was dried

over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was dissolved in acetonitrile. With rapid stirring, several drops of pyridinium hydrogen fluoride were added. The progress of the reaction was monitored with TLC. Precipitate was filtered and washed with diethyl ether. The ether solution was combined with the filtrate liquid and concentrated. The residue was separated by silica gel flash column chromatography using 6:1 hexanes/ethyl acetate to yield 0.42g (68%) of product. ^{1}H NMR ($CDCl_3$) δ 7.78-7.65 (m, 2H), 7.35-7.25 (m, 3H), 6.35 (t, 1 H), 6.05 (d, 2H), 5.9 (s, 1 H), 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.

Compound **19'a,b**: ^{1}H NMR ($CDCl_3$) δ 7.95 (dd, 2H), 7.51 (m, 1H), 7.39 (m, 2H), 6.72 (s, 1H), 6.66 (d, 2H), 6.43 (t, 1H), 5.7 (bs, 1H), 5.0 (m, 2H), 3.91 (s, 3H), 3.85 (s, 3H), 3.75 (s, 6H), 3.3 (m, 3H), 1.80 (s, 3H), 2.12 (s, 2H), 1.65 (s, 3H). HRMS m/z (MH^+) calcd. 566.6387, obsd. 566.6384.

*Steady-State Photolysis of **19a,b***. A 10 □M solution of **19a,b** in methanol was prepared in a 1cm path length quartz cuvette equipped with a septum. The sample was purged with oxygen free argon and irradiated 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 was 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-Park C18 reverse phase.

*Transient Photolysis of **19a,b***. A 10 □M solution of **19a,b** in methanol was prepared in a 1cm path length quartz cuvette equipped with a septum. The sample was purged with oxygen free argon and irradiated at 355 nm using a 10ns pulse from a Nd: YAG laser. Absorbance spectra were then recorded after successive laser shots.

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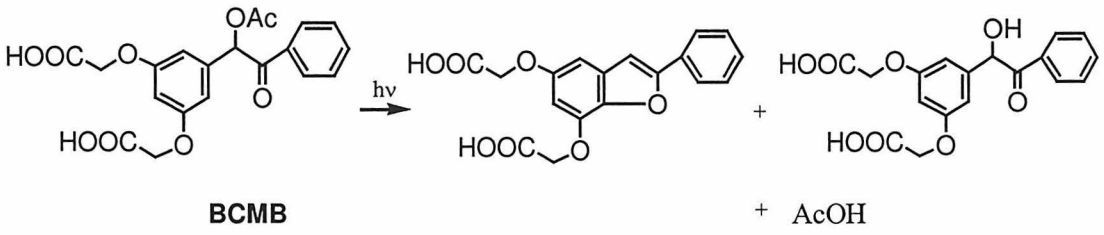
Chapter 4

Water-soluble Bis(carboxymethoxy)benzoin Caged Ubiquinol

Introduction

In order to probe the reaction chemistry of respiratory quinol oxidase enzymes on a rapid time scale, ubiquinol-1 and ubiquinol-2 caged with the 3', 5'-dimethoxybenzoin group were synthesized and studied. However, As we have discussed in chapter 3, poor water solubility of the caged ubiquinols prevented full characterization of the compounds and precluded their use in biological studies.

It has been found that by replacing the methoxy groups with carboxymethoxy groups, the water solubility of the benzoin compound obtained is dramatically increased[3]. *O*-acetyl-3', 5'-bis(carboxymethoxy)benzoin (BCMB) and its photoproducts have been reported to be water-soluble in substantial. Although two photoproducts are formed upon photolysis (Scheme 4.1), one is the expected phenylbenzofuran and the other is 3', 5'-bis(carboxymethoxy)benzoin, indicating that cyclization may be quenched by water with concomitant release of the acetate, photolysis of this cage compound leads to the rapid



Scheme 4.1. Photolysis of BCMB Acetate in Aqueous Solution

release of acetate in high yield. We expected that BCMB-ubiquinol assembly would release ubiquinol in a similar fashion.

Results and Discussion

Synthesis of BCMB Caged Ubiquinol-2

The synthesis followed the similar convergent synthetic route as we described in the synthesis of DMB caged ubiquinol. However, since the carboxymethoxy substituents in the BCMB need to be protected during the ubiquinol coupling reaction and to be deprotected afterwards, reactions for the cleavage of the protecting group has to be mild enough to be non-destructive to the other functional moieties.

Methyl ester and *tert*-butyl ester of BCMB-ubiquinol-2 were synthesized (Figure 4.1). The removal of *tert*-butyl ester protection group was tested with 100%, 50% and

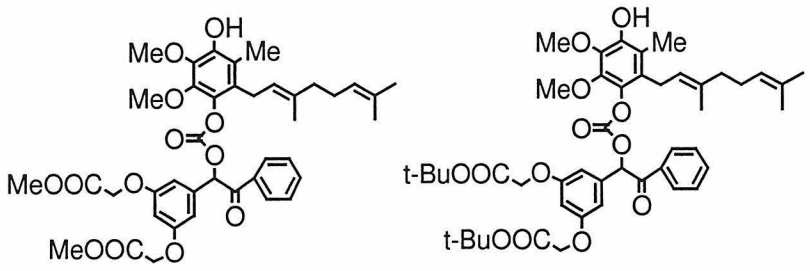


Figure 4.1. Methyl Ester and *tert*-Butyl Ester of BCMB-ubiquinol-2

33% TFA/CH_2Cl_2 solution at both room temperature and 0°C for varied length of time. Thioanisole was also added in the reactions as a nucleophilic scavenger. All of the

reactions led to inseparable mixtures or/and insoluble white precipitates. This was speculated to be due to the protonation of the isoprenoid tail in ubiquinol-2, which then either isomerized to give side product mixtures or polymerized via nucleophilic attack at the formed carbocation. On the other hand, the removal of methyl-ester in Compound B with LiOH/THF/water at 0°C led to the cleavage of carbonate linkage. Another attempt to cleave methyl-ester with BCl_3 at –40°C resulted in a mixture in which none of the separable ingredients is desired product. This could be due to that the isoprenoid unit in the ubiquinol is reactive towards the strong electrophiles.

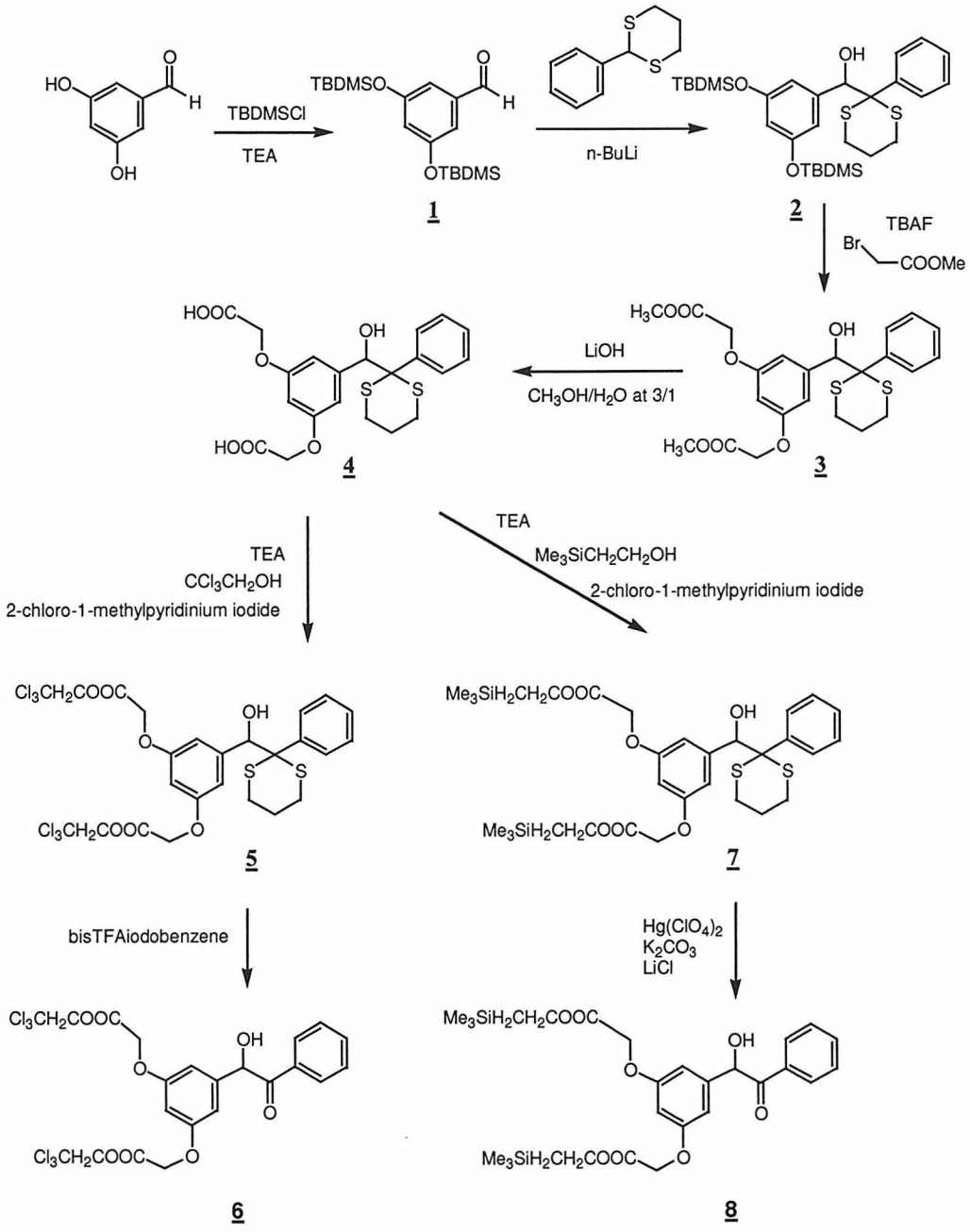
From these results, it is obvious that both strong acidic and basic conditions need to be avoided in the deprotection reaction. In addition, carbonate linkage in the targeted ubiquinol-BCMB adduct could be cleaved under illumination or high temperature[1]. Therefore it is critical to choose an appropriate protecting reagent that can be cleaved under non-invasively mild conditions.

β,β,β-trichloroethanol (Cl_3CCH_2OH) and 2-(trimethylsilyl)ethanol ($Me_3SiCH_2CH_2OH$) were selected to be used as protecting reagents to form ester with the carboxymethoxy substituents in BCMB. β,β,β-trichloroethanol ester can be cleaved by zinc powder in THF aqueous solution. 2-(trimethylsilyl)ethanol esters can be cleaved by HF-pyridine in THF solution. The cleavage conditions for both of the two protecting groups are considered mild enough for the generation of the free carboxylic acid groups.

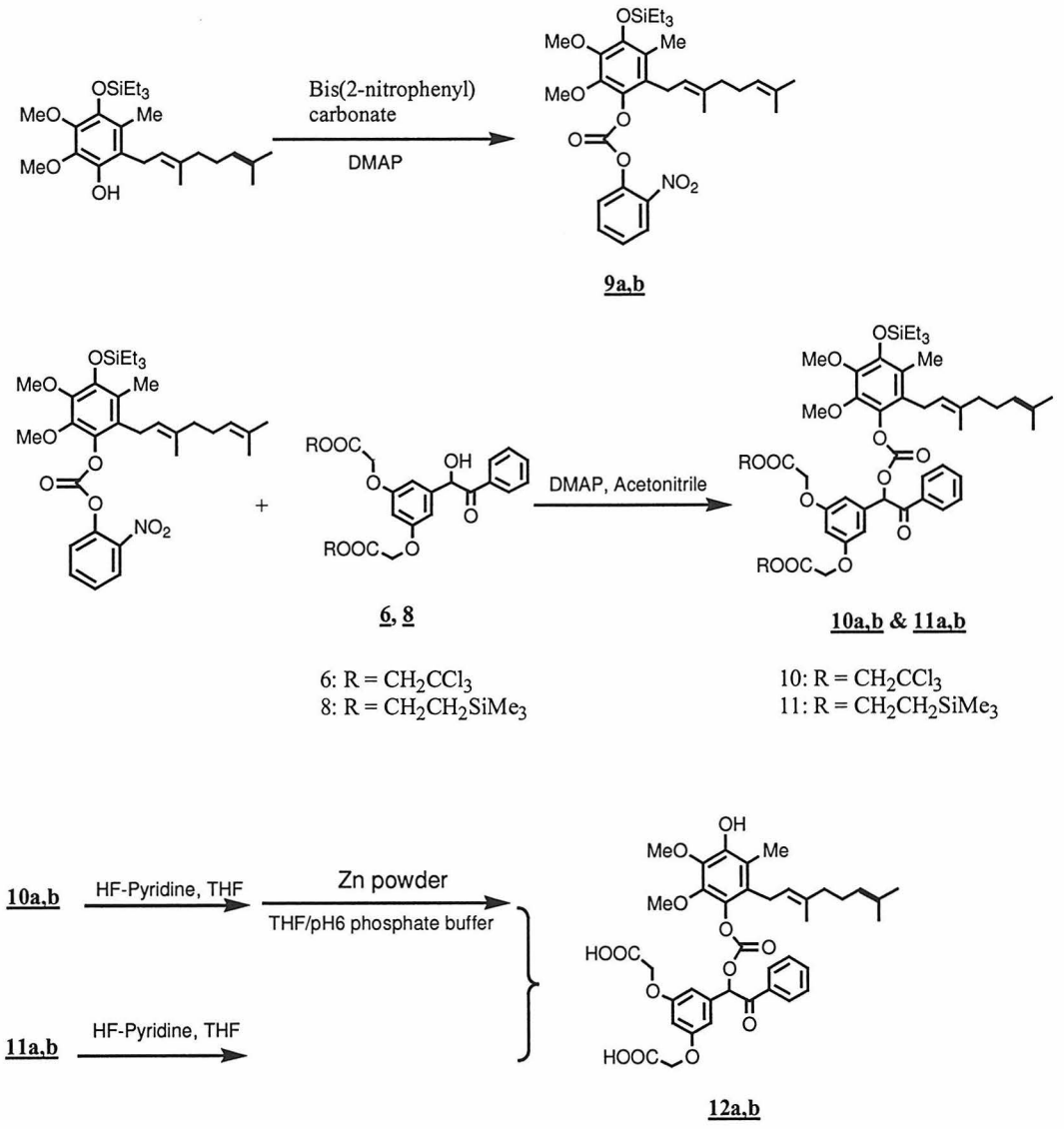
β,β,β-trichloroethanol and 2-(trimethylsilyl)ethanol ester of BCMB were prepared starting from 3,5-dihydroxybenzaldehyde (Scheme 4.2). After protected as TBDMS ether, the benzaldehyde was condensed with phenyl dithiane lithium anion and

then alkylated with TBAF and methyl bromoacetate. The methyl acetate was cleaved with LiOH/MeOH to give free acid and then reacted with Cl_3CCH_2OH or $Me_3SiCH_2CH_2OH$ to form esters. The dithiane was hydrolyzed at the last step to give the protected BCMB **6** and **8**.

Monosilyl ubiquinol – 2 was activated by reacting with bis(2-nitrophenyl)carbonate to form the 2-nitrophenyl carbonate ubiquinol. The coupling reaction between activated ubiquinol and the protected BCMB was catalyzed by 50% DMAP in acetonitrile. **11a,b** was obtained in 12% yield after 5 days' reaction and **10a,b** was obtained in 46% after 3 days' reaction. The low yield of these reactions may be due to the steric hindrance caused by the two flexible, lengthy and bulky substituents on the benzoin blocking the hydroxyl group. **10a,b** was treated with HF-pyridine in THF and then zinc powder in THF and pH 6.5 phosphate buffer solution for 30 minutes to yield **12a,b**. **11a,b** was treated with HF-pyridine for 4 hours to yield the same product.



Scheme 4.2. Synthesis of β,β,β-Trichloroethanol and 2-(Trimethylsilyl)ethanol Ester of BCMB



Scheme 4.3. Assembly of the BCMB Caged Ubiquiunol-2

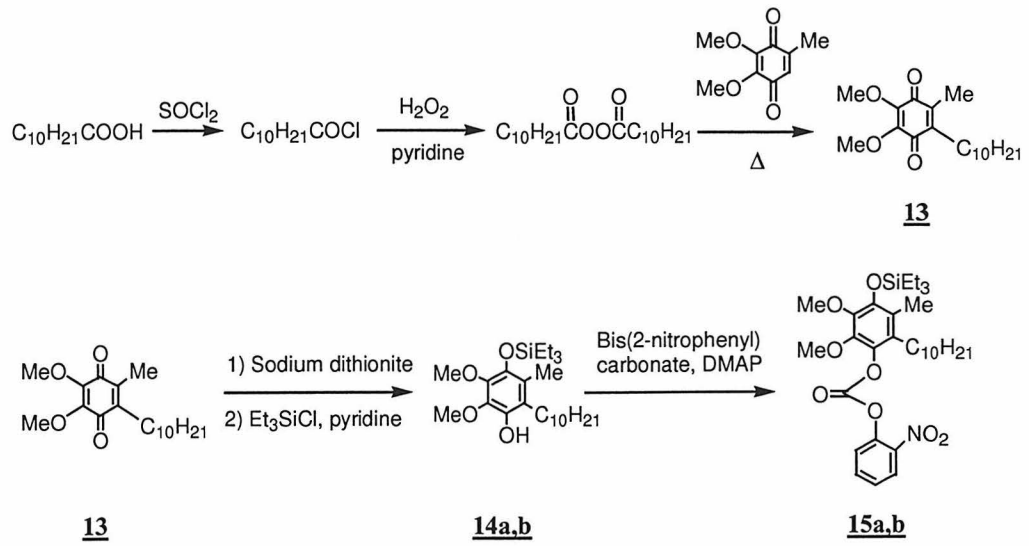
Synthesis of BCMB Caged Decylubiquinol

The complexity of the above synthesis was due to the fragile functional groups in the target molecule **12a,b**: The carbonate linkage would hydrolyze in basic solutions, the

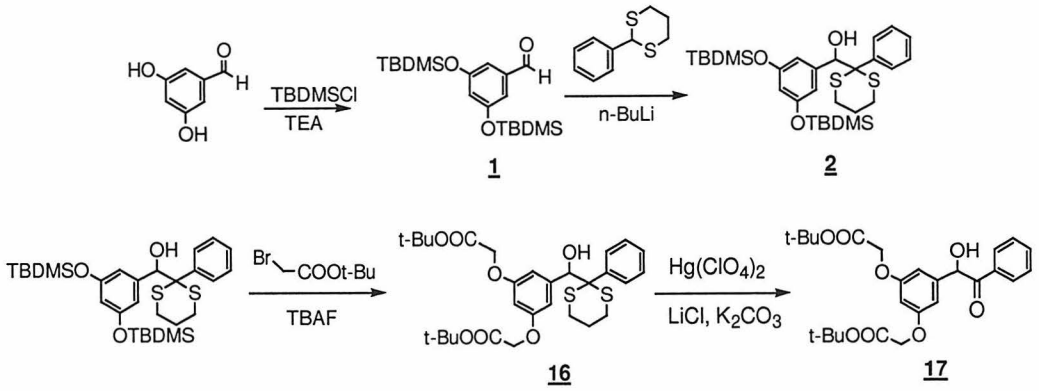
molecule is sensitive to heat and light, the isoprenoid tail on the ubiquinol-2 is unstable under acidic solutions, etc. To develop an alternative for the above synthesis, we replaced ubiquinol-2 with decylubiquinol in our new synthesis. Decylubiquinol is considered as a more robust substitute for physiological ubiquinol[7]. It has been common to use commercially available decylquinol to study the steady state kinetics of quinol oxidizing enzymes. The advantage of using decylubiquinol to replace ubiquinol-2 in this synthesis is that acidic hydrolysis will be allowed for cleavage of ester protecting group.

Decylubiquinone was synthesized following the procedure reported by C.A. Yu[8]: 11-undecanoic acid was converted to undecanoyl chloride by treatment with $SOCl_2$ and was then oxidized to undecanoyl peroxide. The decylubiquinone was obtained by heating a mixture of Q_0 and undecanoyl peroxide in acetic acid solution under argon at 95°C for 14 hours.

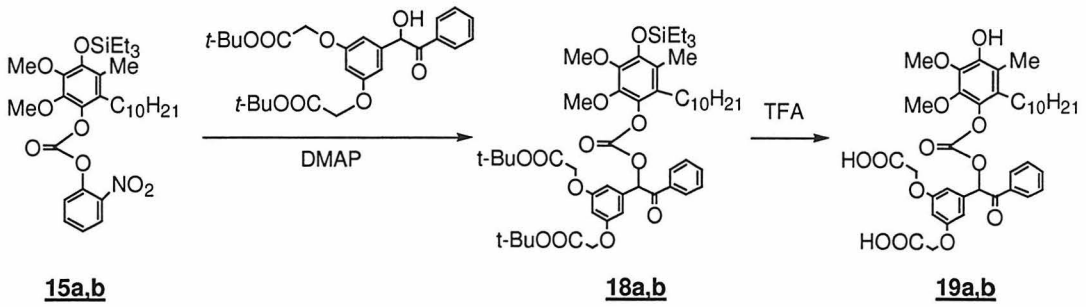
tert-Butyl ester of BCMB was used as a precursor to the BCMB cage since *tert*-butyl ester can be easily cleaved by TFA. It was readily prepared from **2** by reacting with TBAF and *tert*-butyl bromoacetate (Scheme 4.5) following the same procedure as illustrated in Scheme 4.3, except that at the last step TFA/dichloromethane (50%) was used to deprotect carboxylic acid group. BCMB caged decyubiquinol **19a,b** was obtained at a satisfactory overall yield.



Scheme 4.4. Synthesis of Activated Decylubiquinol



Scheme 4.5. Synthesis of the *tert*-Butyl Ester of BCMB



15a,b **18a,b** **19a,b**

Scheme 4.6. Assembly of Caged Decylubiquinol

Steady-state Photolysis of BCMB Caged Decylubiquinol

Figure 4.2 shows the spectra from steady-state photolysis reactions of decylubiquinol-BCMB performed in the following solvents: acetonitrile; 100 mM Na-PO_4, pH 7.4; and 100 mM Na-PO_4, 0.1% DDM, pH 7.4. A growing peak at 288 nm during photolysis indicates the formation of the benzofuran. In aqueous buffer solution without detergent, photolysis of decylubiquinol-BCMB resulted in a broad peak around 276 nm whose intensity is much lower than that observed in the photolysis in acetonitrile. This observation is consistent with the report [3] that the photolysis of BCMB caged compound in aqueous solution gave rise to not only substituted benzofuran, but also the parent caging group BCMB due to the nucleophilic attack of water on the photolysis intermediates. HPLC analysis of the photolysis products revealed the presence of both the substituted benzofuran and BCMB, in a ratio of 30:70. In detergent buffer solutions, photolysis of decylubiquinol-BCMB gave rise to a spectral peak at 284 nm whose

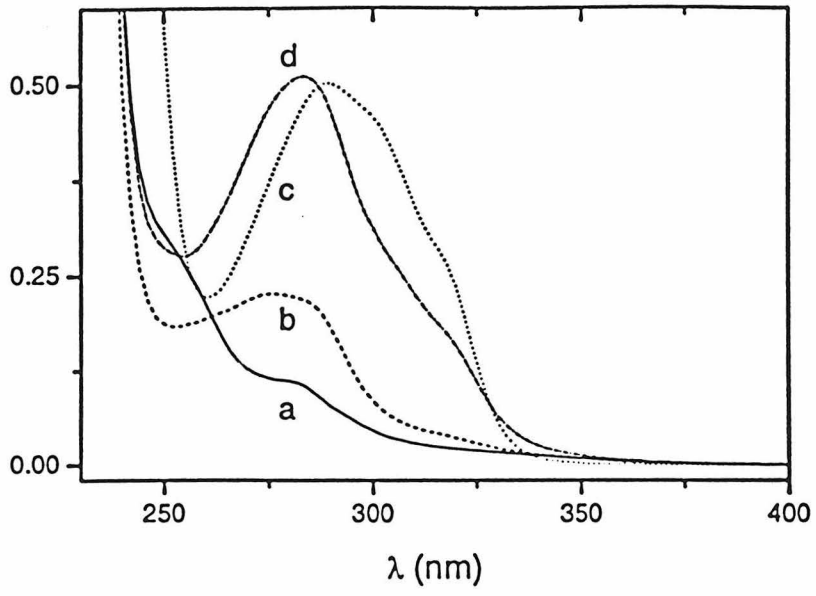


Figure 4.2. The Steady Photolysis of BCMB Caged Decylubiquinol

(a) the spectrum of intact decylubiquinol-BCMB in 100 mM Na-PO_4, 0.1% DDM, pH7.4, (b) the photolysis products of 50uM decylubiquinol-BCMB in 100 mM Na-PO_4, pH7.4, (c) the photolysis products of 50uM decylubiquinol-BCMB in acetonitrile, (d) the photolysis products of 50uM decylubiquinol-BCMB in 100 mM Na-PO_4, pH7.4.

intensity is comparable to that observed in acetonitrile. HPLC analysis showed that the benzofuran photoproduct was formed in >80% yield. The high yield of substituted benzofuran observed in detergent solution suggested that the decylubiquinol-BCMB might be localized in detergent micelles and therefore shielded from water during photolysis.

It was also observed that the BCMB caged decylubiquinol is reasonably soluble in aqueous solution. The presence of two carboxylate groups provides water solubility to the

caged compounds without negatively affecting the photolysis properties of the protecting group. The solubility of DQ-BCMB was measured as > 300 μM in 0.1% DDM solution, a concentration that is sufficiently high for enzymological studies. Moreover, it has the desirable feature of partitioning preferentially into the detergent phase of solution. Localized within a hydrophobic environment, the carbonate linkage between BCMB and quinol is less prone to be hydrolyzed. In addition, the proximity of substrate and the membrane-bound quinol oxidase enzymes of interest will facilitate the reaction chemistry.

Laser Photolysis of BCMB Caged Decylubiquinol

To determine the release rate of the free decylubiquinol, transient absorption spectra of 30 μM DQ-BCMB were taken in 100 mM Na-PO_4, 0.1% DDM, pH 7.4. The change in absorbance at 310 nm was used to monitor the formation of benzofuran photoproduct. An average value for ΔA of approximately 0.1 was observed for a single laser pulse with 2 mJ energy, corresponding to the formation of 10 μM of benzofuran and hence the release of 10 μM decylubiquinol carbonate monoester. Considering this was achieved from 30 μM starting material, the quantum yield of the photolysis is sufficiently high for enzyme studies. As has been observed in 3′, 5′-DMB acetate photolysis, photolysis occurs within the instrument response time, setting a lower bound of 10^6 s^{-1} for benzofuran formation.

In our experiments in which transient absorption spectroscopy was used to monitor the reaction of photoreleased decylubiquinol with quinol oxidase enzymes, it was

found that the rates of enzyme reduction by the released quinol were slower than the enzyme turnover, which suggested that decarboxylation of the substrate was rate-limiting in this process. It should be noted that in the photolysis, the first ground state product is a carbonate monoester. Only through subsequent decarboxylation of the carbonate monoester, free quinol will be released. Therefore, the decarboxylation kinetics needs to be studied in more depth. However, since no significant spectra changes occurred as a result of the release of the free quinol molecule, therefore, the decarboxylation step is difficult to observe.

The photolytically formed ubiquinol carbonate monoester remains deprotonated in normal pH ranges. Upon decarboxylation, the quinol anion will take up a proton if the solution pH is lower than the quinol pKa. Thus, upon photolysis of the caged decylubiquinol, there will be a rapid acidification of the solution and a subsequent alkalinization that should occur at the rate of decarboxylation of the quinol carbonate monoester. This analysis suggests that time-resolved absorption spectroscopy in conjunction with pH indicators could be used in measuring the decarboxylation rate.

Based on the above considerations, laser photolysis reactions were performed in aqueous solution in the presence of pH indicator dyes, so that the pH changes during the reaction could be measured by the absorbance change of the indicator. Measurements were performed both in the presence and in the absence of 0.1% DDM, in the pH range of 3.9-9.6. The indictor dyes used, along with the observation wavelength for pH change in water and 0.1% DDM, respectively, were as follows: bromphenol blue (pKa = 4.0, λ = 590, 596 nm); bromcresol green (pKa=4.7, λ = 610, 616); methyl red (pKa = 4.8, λ =

522, 522 nm); bromcresol purple (pKa = 4.8, λ = 588, 592 nm); bromthymol blue (pKa = 7.0, λ = 614, 620 nm); m-cresol purple (pKa = 8.3, λ = 578, 578 nm) and phenolphthalein (pKa=9.5, λ = 552, 552 nm).

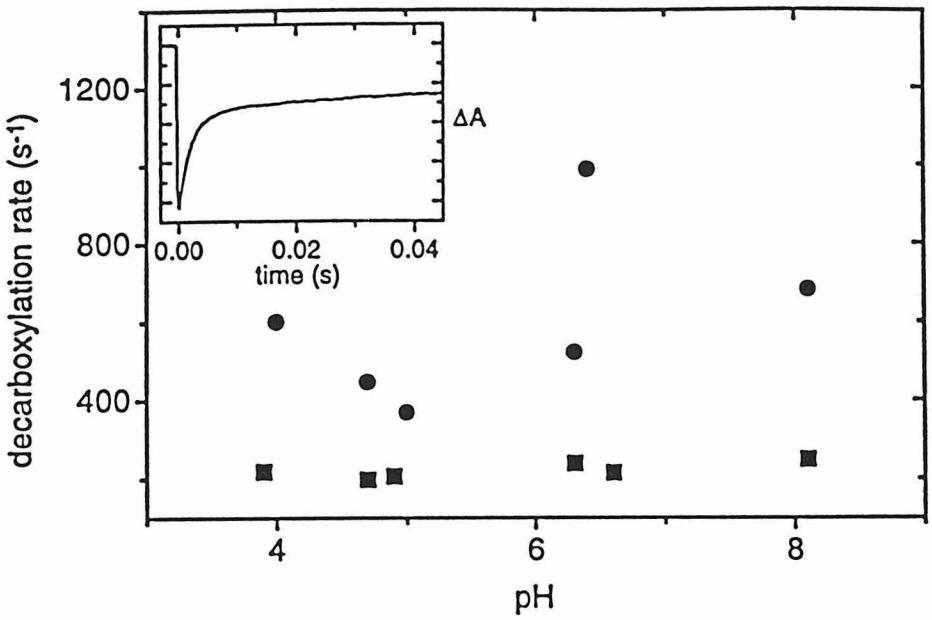


Figure 4.3. Rates of Decarboxylation of the Carbonate Monoester of Decylubiquinol in the Presence (circles) or Absence (squares) of 0.1% DDM as a Function of pH

Inset: transient absorption spectrum of the photolysis of 32 uM BCMB caged decylubiquinol in 11 uM bromocresol purple, 0.1% DDM, pH 6.3.

Figure 4.3 summarizes the results of these measurements. The inset shows a typical absorption transient following the photolysis of BCMB caged decylubiquinol in 0.1% DDM in the presence of bromcresol purple. The rapid decrease in absorbance following the laser flash indicated an acidification of the solution. This was followed by a biexponential rise corresponding to realkalinization of the solution. At all pH values, the

acidification was instantaneous on the time scale of the experiment. In the absence of detergent, the alkalinization reaction occurred over the pH range with first order rate or base-assisted. The more rapid decarboxylation in detergent solution could be explained considering the caged quinol molecules are localized within detergent micelles, an environment that would tend to destabilize the charged carbonate formed upon photolysis. From the above observations, apparently, the rate-limiting step for release of active quinol is the decarboxylation of the quinol carbonate monoester. Sauers et al. have found an empirical relationship between the pKa of aliphatic alcohols and the rate of decarboxylation of the corresponding alkyl carbonates[5]. Rossi and Kao[4] have applied the above relationship to o-nitromandelyloxycarbonyl caged 2,5-di(tert-butyl)hydroquinone and they calculated an approximate $t_{1/2}$ for the decarboxylation of the carbonate of 5 ms. Papageorgiou and Corrie[2] have also reported a $t_{1/2}$ of 4.5 ms for the decarboxylation of a carbamate that was generated photochemically. The values reported herein are in line with those discoveries.

Through the use of control reactions, we have also demonstrated that the BCMB caged ubiquinols are inactive towards quinol oxidase enzymes before photolysis. Reduction of the cytochrome bc_1 was studied following photolysis of the caged decylubiquinol. The electron transfer from decylubiquinol to the hemes was monitored by following the absorption changes at 430 nm. Reduction of the hemes was observed to follow a kinetic phase. The by-products of the photolysis reaction, the substituted phenylbenzofuran and CO_2, are not reactive and neither product has shown inhibition of enzyme activity. The observed kinetics data was fitted with a single-exponential and the

rate constant was found to be 150 s^{-1}. A recent study[6] of the reaction of menaquinol with cytochrome bc_1 monitored by stopped-flow optical spectroscopy reported a b heme reduction rate constant of 1.3 x 10^6 $M^{-1}s^{-1}$ (k_{obs}=13 s^{-1} for 10 μM menaquinol), a rate slower than what was achieved herein with decylubiquinol.

Summary

Photoreleasable ubiquinols were synthesized by coupling ubiquinols with the water-soluble protecting group 3', 5'-bis(carboxymethoxy)benzoin (BCMB) through a carbonate linkage. The resulting compounds possessed reasonable solubility in aqueous solution and showed no reactivity with quinol oxidase enzymes prior to photolysis. The photochemical properties of BCMB caged decylubiquinol were characterized. In detergent solution, quinol release rates as high as 990 s^{-1} were observed, which are comparable to or better than traditional mixing techniques. The photorelease of BCMB caged ubiquinols can serve as a complementary technique to more traditional methods, and has potential use in situations where the turbulent mixing associated with stopped flow may disrupt the integrity of proteins or liposomes. The synthesis developed herein provides a general synthetic protocol for preparing water-soluble BCMB caged compounds.

However, a caged ubiquinol with a release rate on the submillisecond time scale, preferably microsecond time scale, was still not accomplished. Further research is still needed.

Materials and Methods

General: Anhydrous THF was prepared by refluxing over sodium metal and benzophenone. Anhydrous acetonitrile, methylene chloride and pyridine were purchased from Fluka. All other solvents were of reagent grade. ^{1}H NMR spectra were recorded on a GE QE300 spectrometer operating at a nominal frequency of 300 MHz. The optical absorption was monitored with a HP 8452 diode array spectrophotometer. HPLC analysis was performed using a Shimadzu LC-6A system equipped with a Vydac C-18 reverse-phase column. Cytochrome bc_1 from bovine heart was a gift from Dr. C. A. Yu of the department of Biochemistry, Oklahoma State Univeristy. Steady-state photolysis was performed with an Oriel 66011 Hg vapor arc lamp operating at 450 W and filtered through water-cooled Schott glass type UG11 and WG320 filters. Laser spectroscopy was performed with a Lambda Physik LPX201I XeCl excimer laser (308 nm), with its energy in the range of 1.7-2.0 mJ/pulse.

*Synthesis of **1***: 3,5-hihydroxybenzaldehyde (2g, 14.48 mmol) was dissolved in 100mL dry THF and cooled to 0°C. *Tert*-butyldimethylsilyl chloride (5.464 g, 36.24 mmol) was added, followed by triethylamine (5mL, 35.81mmol) dropwise. The solution was stirred at room temperature for overnight. Solvent was removed under reduced pressure. The oil was diluted in diethyl ether and was washed by NH_4Cl aqueous solution. The organic phase was collected and dried with magnesium sulfate. The solution was filtered through a plug of neutral alumina and decolored with active charcoal. The solvent was removed under reduced pressure and the colorless oil was dried *in vacuo*. Yield 4.91g (92%). ^{1}H

NMR ($CDCl_3$) δ 9.86 (s, 1H), 6.96 (d, 2H), 6.59 (t, 1H), 0.99 (s, 18 H), 0.22 (s, 12H). MS (FAB) m/z (MH^+) calcd 367.21, obsd 367.22.

Synthesis of ***2***: 2-phenyl-1,3-dithiane (6.28g, 32 mmol) was dissolved in dry THF and the solution was cooled to 0°C under argon flow. *n*-Butyllithium (2.5 M in hexanes) was added. The solution was stirred at 0°C for 30 minutes and 10mg 1,10-phenanthroline was added as titration indicator. Compound **1** was added dropwise until the red color vanished. The solution was stirred for 1 hr at 0°C then poured into 0.1N HCl aqueous solution and extracted with dichloromethane. The organic phase was washed with brine, dried with magnesium sulfate, decolored with active charcoal, filtered through a plug of silica gel and dried under reduced pressure. The resulting oil was crystallized from ethanol/water to give a white powder. Yield 16.55g (92%). ^1H NMR ($CDCl_3$) δ 7.71(m, 2H), 7.30 (m, 3H), 6.176(t, 1H), 5.998 (d, 2H), 4.868 (s, 1H), 2.71 (m, 4H), 1.92 (m, 2H), 0.920 (s, 18H), 0.094 (s, 12H). MS (FAB) m/z (MH^+) calcd 563.25, obsd 563.25.

Synthesis of ***3*** *and* ***16***: A solution of **2** (11.24g, 20 mmol) and *tert*-butyl bromoacetate (4.27g, 22 mmol) (for **3**, methyl bromoacetate was used instead) were dissolved in dry THF under an argon flow. The solution was cooled to 0°C and treated with 22mL 1M TBAF in THF dropwise. The solution was allowed to react overnight, then was poured into ethyl acetate and washed with water. The organic phase was dried with magnesium sulfate and solvent was removed under reduced pressure. The residue was dissolved in diethyl ether and filtered through a neutral alumina plug and decolored by active charcoal

and dried *in vacuo*. The product was crystallized from ethyl acetate/hexanes to form a white power.

3: Yield 8.89g (93%). ^{1}H NMR ($CDCl_3$) δ 7.697, 7.672 (dd, 2H), 7.30 (m, 3H), 6.385(t, 1H), 6.023 (d, 2H), 4.911 (s, 1H), 4.356 (s, 4H), 3.781 (s, 6H), 2.724 (m, 4H), 1.926 (m, 2H), MS (FAB) m/z (MH^+) calcd 479.12 , obsd 479.14.

16: Yield 10.34g (92%). ^{1}H NMR ($CDCl_3$) δ 7.715-7.688 (dd, 2H), 7.293 (m, 3H), 6.367(t, 1H), 5.993 (d, 2H), 4.900 (s, 1H), 4.217 (s, 4H), 2.708 (m, 4H), 1.915 (m, 2H), 1.470 (s, 18H), MS (FAB) m/z (MH^+) calcd 563.21, obsd 563.20.

Synthesis of 4: A solution of **3** (4.8g, 10mmol) in 120mL of 3/1 (v/v) methanol/water was cooled to 0°C in ice bath. The solution was treated with LiOH (2g, 100mmol) and stirred at 0-10°C for 18 hours. Methanol was removed under reduced pressure. The solution was acidified to pH 2-3, extracted several times with ethyl acetate. The organic phase was collected and washed with brine, dried with magnesium sulfate and solvent was removed *in vacuo*. The product was crystallized from ethyl acetate/diethyl ether/hexanes to afford a white power. Yield 3.64g (81%), ^{1}H NMR (acetone-d_6) δ 7.764, 7.737 (dd, 2H), 7.296 (m, 3H), 6.348 (t, 1H), 5.999 (d, 2H), 4.982 (s, 1H), 4.421 (s, 4H), 2.71 (m, 4H), 1.88 (m, 2H). MS (FAB) m/z (MH^+) calcd 451.09, obsd 451.12.

Synthesis of 5 and 7: To a methylene chloride (8mL) suspension of 2-chloro-1-methyl pyridinium iodide (3.14g, 11.92mmol) was added a mixture of **4** (1.78g, 3.97mmol), 2-(trimethylsilyl)ethanol (1.42g, 12mmol) (use trichloroethanol for **5**), and triethylamine

(2.5g, 24.7mmol) in methylene chloride (10mL). After it was stirred overnight, the mixture was quenched with water and the organic layer was separated. The aqueous layer was extracted several times with ether and the combined organic layers were dried over sodium sulfate. The solvent was removed *in vacuo* and the product was purified by silica gel column chromatography by using hexanes-ethyl acetate (4/1) as the eluting solvent.

5: Yield 2.08g (74%). ^{1}H NMR ($CDCl_3$) δ 7.94 (dd, 2H), 7.32 (m, 3H), 6.444(t, 1H), 6.045 (d, 2H), 4.910 (s, 1H), 4.827 (s, 4H), 4.504 (s, 4H), 2.71 (m, 4H), 1.93 (m, 2H), MS (FAB) m/z (MH^+) calcd 710.92, obsd 710.95.

7: Yield 2.01g (78%). ^{1}H NMR ($CDCl_3$) δ 7.688 (dd, 2H), 7.295 (m, 3H), 6.386(t, 1H), 6.013 (d, 2H), 4.911 (s, 1H), 4.306 (s, 4H), 4.253 (t, 4H), 2.70 (m, 4H), 1.925 (m, 2H), 1.030 (t, 4H), 0.051 (s, 18H). MS (FAB) m/z (MH^+) calcd 651.23, obsd 651.29.

Synthesis of ***6***: Bis(trifluoroacetoxy) iodobenzene (1.29g, 3mmol) dissolved in 2mL of CH_3CN was added into a solution of **5** (1.42g, 2mmol) in 10mL of 9/1 CH_3CN/water. After the reaction was completed as monitored by TLC (about 10 mins), the solution was poured into saturated aqueous sodium bicarbonate and extracted several times with ethyl acetate. The organic extracts were combined and dried over magnesium sulfate. The solvent was removed in *vacuo* and the residue was purified by silica gel column flash chromatography using hexanes-ethyl acetate (3/1) as the eluting solvent. Yield 1.01g (81%). ^{1}H NMR ($CDCl_3$) δ 7.913 - 7.881 (dd, 2H), 7.560 (m, 1H), 7.425 (t, 2H), 6.565 (d, 2H), 6.449(t, 1H), 5.846 (s, 1H), 4.805 (s, 4H), 4.715 (s, 4H) MS (FAB) m/z (M^+) calcd 619.91, obsd 619.92.

*Synthesis of **8** and **17***: To a solution of **7** or **16** (3mmol) in 30ml THF was added LiCl (3 mmol) and cooled to 0°C. Mercuric perchlorate (6mmol) dissolved in 3ml water was added and the solution was stirred for 20 mins. Potassium carbonate (12.1mmol) aqueous solution was added dropwise to adjust the pH of the solution to about 7-9. The solution was extracted several times with ethyl acetate and the organic layers were combined to dry over magnesium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography using hexanes-ethyl acetate (3/1) as the eluting solvent.

8: Yield 1.63g (97%). ^{1}H NMR ($CDCl_3$) δ 7.85 (dd, 2H), 7.56 (m, 1H), 7.41 (m, 2H), 6.524 (d, 2H), 6.387 (t, 1H), 5.840 (s, 1H), 4.505 (s, 4H), 4.270 (t, 4H), 1.012 (t, 4H), 0.050 (s, 18H), MS (FAB) m/z (M^+) calcd 560.23 , obsd 560.29.

17: Yield 1.39g (98%). ^{1}H NMR ($CDCl_3$) δ 7.912, 7.887 (dd, 2H), 7.534 (m, 1H), 7.426 (m, 2H), 6.502 (d, 2H), 6.374(t, 1H), 5.823 (s, 1H), 4.420 (s, 4H), 1.458 (s, 18H). MS (FAB) m/z (M^+) calcd 472.21, obsd 472.22.

*Synthesis of **14a,b***: A round-bottom flask equipped with stir bar is charged with decylubiquinone (0.32g, 1mmol) and 10mL 4/1 diethyl ether/water. With vigorous stirring, sodium dithionite (1.6mmol) was added. The reddish color of the decylubiquinone quickly dissipated and became colorless after 10 mins. The resultant slurry was extracted with hexanes and filtered through a small plug of magnesium sulfate. Solvent was removed in *vacuo* and further dried under high vacuum. The nearly colorless

oil was redissolved in anhydrous acetonitrile. Anhydrous pyridine (1mmol) was added under argon flow. Chlorotriethylsilane (1mmol) was added dropwise using a syringe pump over 5 hours. The solution was allowed to react for another 1 hour. Solvent and excess TES chloride was removed *in vacuo* and the product was purified by silica gel column flash chromatography using hexanes-ethyl acetate (9/1) as the eluting solvent to give a colorless oil. Yield 0.28g (66%). ^{1}H NMR ($CDCl_3$) δ 5.370 (s, 1H), 3.887 (s, 3H), 3.762 (s, 3H), 2.569 (t, 2H), 2.107 (s, 3H), 1.442 (m, 4H), 1.256 (m, 14H), 0.950 (t, 9H), 0.874 (t, 3H), 0.743 (q, 6H).

*Synthesis of **9a,b** and **15a,b***: A solution of ubiquinol silyl ether (2 mmol) and 2-nitrophenyl carbonate (1.22g, 4mmol) was prepared in 50mL anhydrous methylene chloride. To this solution, 1mmol DMAP was added. The solution was stirred under argon for 5 hours. The solvent was removed *in vacuo* and ether was added to the residue. The solution was poured into 0.01N HCl aqueous solution and extracted with ether for a few times. The product was purified by silica gel column flash chromatography using hexanes-ethyl acetate (4/1) as the eluting solvent.

9 a,b: Yield 1.16g (97%). ^{1}H NMR ($CDCl_3$) δ 8.22 (d, 1H), 7.697 (t, 1H), 7.439 (t, 2H), 3.911 (s, 3H), 3.776 (s, 3H), 3.490 (m, 2H), 2.569 (d, 2H), 2.107 (s, 3H), 1.852 (m, 4H), 1.431 (s, 3H), 1.253 (s, 3H), 0.956 (s, 3H), 0.865 (t, 9H), 0.763 (q, 6H) MS (FAB) m/z (MH^+) calcd 600.30, obsd 600.36.

15a,b: Yield 1.15g (96%). ^{1}H NMR (acetone-d_6) δ 8.219 (dd, 1H), 7.697 (t, 1H), 7.439 (t, 2H), 3.911 (s, 3H), 3.776 (s, 3H), 2.623 (t, 2H), 2.143 (s, 3H), 1.417 (m, 2H), 1.253 (m, 14H), 0.956 (t, 9H), 0.865 (t, 3H), 0.763 (q, 6H).

*General procedure for the synthesis of **10a,b**, **11a,b** and **18a,b***:

The activated silylether of ubiquinol (**9a,b** or **15a,b**) 1mmol and benzoin (**6**, **8** or **17**) 1.2mmol were dissolved in 50 ml anhydrous methylene chloride. DMAP (60mg, 0.5mmol) was added. The solution was stirred under argon for one day and then TLC was used to monitor the progress of the reaction. When the reaction completed, solvent was removed under reduced pressure. The product was purified by silica gel column flash chromatography using hexanes-ethyl acetate (6/1) as the eluting solvent.

10a,b: Yield 53% after reacting for 3 days. ^{1}H NMR ($CDCl_3$) δ 7.89 (m, 2H), 7.52 (m, 1H), 7.38 (m, 2H), 6.71 (d, 2H), 6.689 (t, 1H), 6.472 (s, 1H), 5.062-4.957 (m, 2H), 4.785(s, 4H), 4.697 (s, 4H), 3.798 (m, 5H), 3.750(s, 3H), 2.080 (s, 3H), 2.056-1.960 (bm, 4H), 1.710 (s, 3H), 1.641 (s, 3H), 1.558 (s, 3H), 0.943 (t, 9H), 0.743(t, 6H) MS (FAB) m/z (M^+) calcd 1080.18, obsd 1080.27.

11a,b: Yield 12% after reacting for 5days. ^{1}H NMR ($CDCl_3$) δ 7.90(m, 2H), 7.52 (m, 1H), 7.39 (m, 2H), 6.73 (d, 2H), 6.687 (t, 1H), 6.475 (m, 1H), 5.062-4.957 (m, 2H), 4.540(s, 4H), 4.28 (t, 4H), 3.80 (m, 5H), 3.75(s, 3H), 2.08(s, 3H), 2.05-1.97 (bm, 4H), 1.71(s, 3H), 1.64 (s, 3H), 1.56 (s, 3H), 1.03 (t, 4H), 0.94 (t, 9H), 0.74(t, 6H), 0.042 (s, 18H), MS (FAB) m/z (M^+) calcd 1020.49, obsd 1020.58.

18a,b: Yield 73%. ^{1}H NMR ($CDCl_3$) δ 7.912 (dd, 2H), 7.509 (m, 1H), 7.383 (m, 2H), 6.711 (d, 2H), 6.686 (s, 1H), 6.466 (t, 1H), 4.462 (s, 4H), 3.786 (s, 3H), 3.744 (s, 3H), 2.524 (m, 2H), 2.106 (s, 3H), 1.473 (m, 18H), 1.367 (m, 2H), 1.249 (m, 14H), 0.942 (t, 3H), 0.870 (t, 9H), 0.729 (q, 6H).

*Synthesis of **12a,b** from **10a,b***: A flask equipped with a stir bar was charged with 10 mL of acetonitrile and **10a,b** (324mg, 0.3mmol) with rapid stirring, several drops of pyridinium hydrogen fluoride was added. The reaction was monitored by TLC and solvent was removed *in vacuo* when all the starting material has been converted to product. The residue was extracted with diethyl ether. The combined ether solution was filtered through a plug of anhydrous $MgSO_4$ and solvent was removed *in vacuo*. The resulting solid was dissolved in 10 mL THF and cooled to 0°C in an ice bath. Zinc dust (0.5g), followed by 1M pH 6.5 phosphate aqueous buffer (2mL) were added to the rapidly stirred solution. Ice bath was removed and the solution was allowed to react for 30 mins. Zinc dust was filtered off and the filtrate was poured to saturated NH_4Cl solution and extracted with diethyl ether. The ether solution was concentrated and the product was purified by gradient HPLC with 0.1% TFA containing CH_3CN and water as eluting solvent. Yield: 79mg (37%). ^{1}H NMR ($CDCl_3$) δ 8.09 (m, 2H), 7.61 (m, 1H), 7.48 (m, 2H), 7.00 (s, 1H), 6.89 (d, 2H), 6.58 (t, 1H), 4.98 (m, 2H), 4.73 (s, 4H), 3.72 (s, 3H), 3.68 (s, 3H), 3.26 (m, 2H), 2.18 – 1.84 (bm, 7H), 1.65 (m, 3H), 1.54 (m, 6H). MS (FAB) m/z (MH^+) calcd 707.27, obsd 707.43.

*Synthesis of **12a,b** from **11a,b***: To a rapidly stirred solution of **11a,b** (210mg, 0.2mmol) in 10 mL CH_3CN, several drops of pyridinium hydrogen fluoride were added. Progress of the reaction was monitored by TLC until all the spots were below R_f 0.2 in 4/1 hexanes/ethyl acetate eluting solvent. 10 mL of saturated NH_4Cl aqueous solution was added and the solution was stirred for another 10 mins. The mixture was extracted several times with ethyl acetate. The organic extracts were combined and filtered through a small plug of magnesium sulfate and evaporated in *vacuo*. The resulting solid was subjected to gradient HPLC purification with 0.1% TFA containing CH_3CN and water as eluting solvent. Yield 34mg (24%).

*Synthesis of **19a,b***: **18a,b** (280mg) was added to 10 mL 10/10/1 TFA/CH_2CH_2 /water solution under stirring. Progress of the reaction was monitored by TLC until all the starting material converted to the product. The solvent was removed *in vacuo*. Product was purified with HPLC purification with 0.1% TFA containing CH_3CN and water as eluting solvent. Yield 195mg (91%). ^1H NMR ($CDCl_3$) δ 8.086 (m, 2H), 7.604 (m, 1H), 7.487 (m, 2H), 7.004 (s, 1H), 6.887 (d, 2H), 6.585(t, 1H), 4.726 (s, 4H), 3.781 (s, 6H), 2.557(m, 2H), 2.134 (s, 3H), 1.434(m, 2H), 1.278 (m, 14H), 0.859 (t, 3H). ^{13}C NMR (Acetone-d_6) δ 193.194, 169.230, 160.173, 146.897, 135.025, 134.090, 129.802, 129.204, 129.157, 108.730, 102.660, 80.708, 65.180, 60.612, 60.488, 32.190, 27.089, 22.856, 13.880, 11.050.

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Chapter 5

Synthesis of N-hydroxy-2-thiopyridone Caged Ubiquinol and Synthesis of Ubiquinol Analogues Attached with a Carboxylic Acid Group

Introduction

In order to study rapid electron transfer in quinol oxidizing enzymes, we have prepared the photoreleasable ubiquinols based on the photochemistry of 3', 5'-dimethoxybenzoin. However, these compounds failed to photolytically generate free ubiquinol in sub-millisecond time scale.

In a ubiquinol molecule, hydroxyl groups are the only active functionality that can be used in caging reactions. All of the compounds that we have prepared share a common structural feature: the caging group is linked to the hydroxyl group in quinol. Consequently, a carbonate linkage had to be used in coupling the caging groups and the substrate. However, if a ubiquinol molecule is attached with a carboxylic acid group and still possesses similar enzymatic activities and binding behaviors relative to the native substrate ubiquinol, then such an analogue can be used in enzymatic studies. Such a substrate can be readily caged by BCMB via an ester bond, instead of the carbonate linkage. The bulky and hydrophilic BCMB will block the its access to the quinol binding sites in the protein and therefore will render the compound biologically inactive. Upon photolysis, the biologically active ubiquinol analogue will be released and enzymatic electron transfer will be triggered on.

In such a design, an obvious risk is that upon attaching ubiquinol with a carboxylic acid group, the resultant derivatives may completely lose ubiquinol enzymatic activities or may have different partition behaviors or different electron transfer kinetics compared with native ubiquinol. However, it is possible that by maximally retaining the structural features of native ubiquinol and finely tuning the position of the added

carboxylate functionality, a biologically active analogue that maintains the same reactivity as that of the native ubiquinol might be attainable.

On the other hand, although study of the structural requirement for quinol/quinone in quinol/quinone-mediated electron transfer is essential for elucidation of the binding site and the reaction mechanism, the interactions between quinol/quinone and protein are not yet fully understood[4]. The analogues herein prepared can be used for obtaining more understanding on this aspect. Particularly, since the carboxyl group can be readily derivatized, different biophysical probes, such as fluorophores, spin labels etc., can be attached to the carboxyl group so that many interesting biophysical studies might be performed.

Due to the lack of crystal structure coordinates, a structural modeling could not be performed yet. Therefore, a series of such ubiquinol analogues were prepared. Carboxylic acid group was created at the 2-, 3-, 5- or 6- position respectively (Figure 5.1) with various linker lengths. The syntheses developed herein were designed to be versatile and general enough to allow for facile preparations of other derivatives via the similar synthetic protocol. For example, for a carboxylic acid group attached to 2-position of ubiquinone, linkers with different lengths (carbon numbers) should be obtainable following the same synthesis scheme. This ensures that a number of quinol analogues can be generated through a reliable synthesis strategy. All of these analogues will then be subject to biological activity screenings in cytochrome bc_1 and cytochrome bo_3.

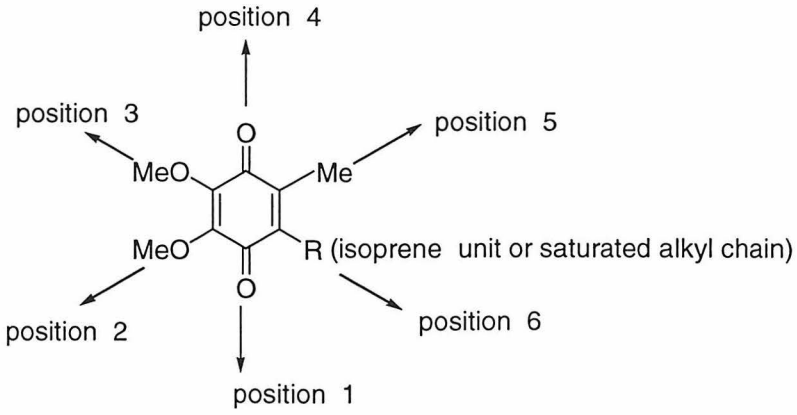
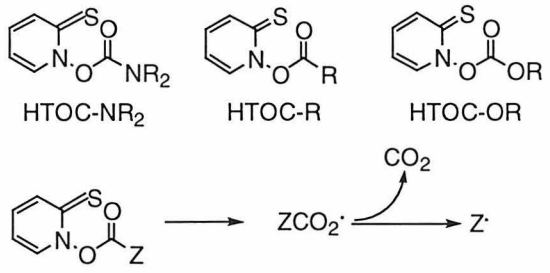


Figure 5.1. The Different Positions in Ubiquinone Where a Carboxylate Functionality can be Introduced

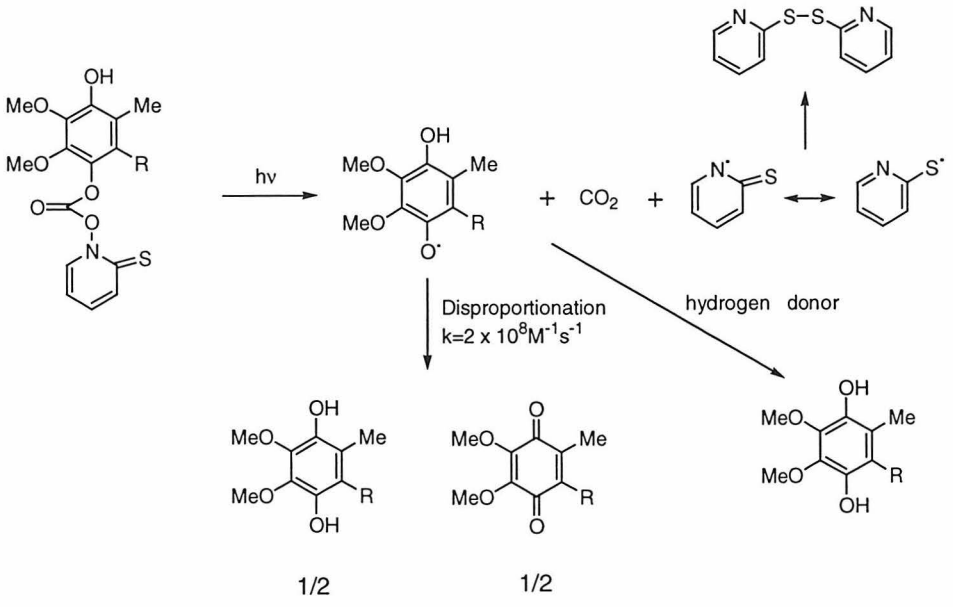
In the mean time, another strategy to improve the ubiquinol release rate, N-hydroxy-2-thiopyridone caged ubiquinol, was also pursued. Upon photolysis of the N-hydroxy-2-thiopyridone derivatives, HTOC-NR_2, HTOC-R and HTOC-OR, cleavage of the N-O bond generates carbamoyloxy, acyloxy or alkoxycarbonyloxy radicals respectively[2] (Scheme 5.1). In general, rapid decarboxylation (> 10^8 s^{-1}) occurs when the radical thus formed is stable [9,10].



Scheme 5.1. Photolysis of N-hydroxy-2-thiopyridone Derivatives

It is expected that when ubiquinol is linked with the N-hydroxy-2-thiopyridone via a carbonate linkage, a quinoxycarbonyloxy radical will be produced upon photolysis. Through subsequent rapid decarboxylation, semiubiquinone will be generated. In the absence of an efficient hydrogen donor, the semiubiquinone will disproportionate to form one equivalent ubiquinol and one equivalent ubiquinone. If an efficient hydrogen donor is added to the reaction and the proton donating reaction is fast enough, it will compete with the disproportionation reaction, directing the overall reaction to the generation of ubiquinol (Scheme 5.2).

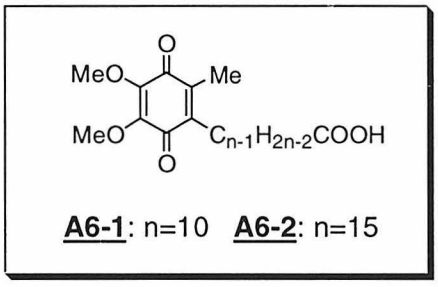
In this chapter, synthesis of N-hydroxy-2-thiopyridone caged ubiquinol and synthesis of ubiquinol analogues attached with a carboxylic acid group are described. These compounds provide new opportunities for achieving our goal of rapid photorelease of two-electron donors.

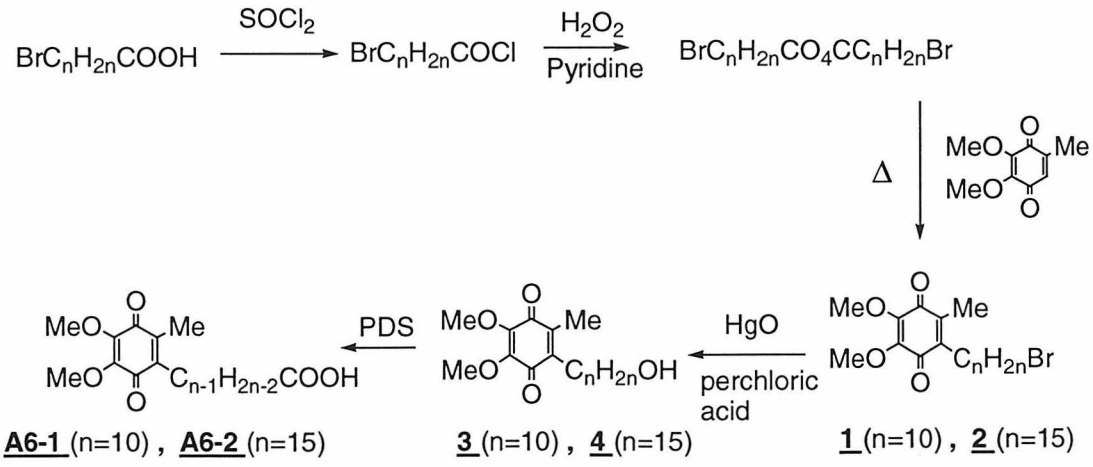


Scheme 5.2. Reaction Pathways of N-hydroxy-2-thiopyridone Caged Ubiquinol

Results and Discussions

Synthesis of Ubiquinol Analogues Attached with a Carboxylic Acid Group at 6-Position. Two analogues with different linker lengths between the carboxylate and the quinone ring, **A6-1** and **A6-2**, were synthesized by a radical coupling reaction between 2,3-dimethoxy-5-methyl-benzoquinone and di-alkanoyl peroxide[12].





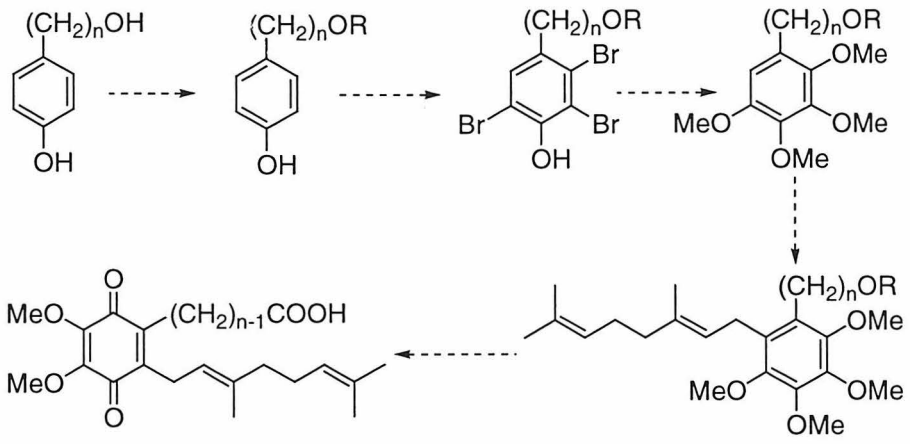
Scheme 5.3. Synthesis of Analogues Attached with a Carboxylic Acid Group at 6-Position

First, $BrC_nH_{2n}COOH$ was treated with thionyl chloride to convert to the corresponding acylchloride. The acylchloride was oxidized with hydrogen peroxide in the presence of pyridine to afford di-alkanoyl peroxide in a quantitative yield. The peroxide was allowed to react with 2,3-Dimethoxy-5-methyl-benzoquinone at 95°C under argon flow to yield ubiquinone bromide **1** and **2**. The bromide was converted to the corresponding alcohol **3** and **4** by mercuric-assisted solvolysis[8] under the action of mercury oxide and perchloric acid. At the last step, PDS oxidation[3] of the alcohol led to the targeted carboxylic acid **A6-1** and **A6-2**.

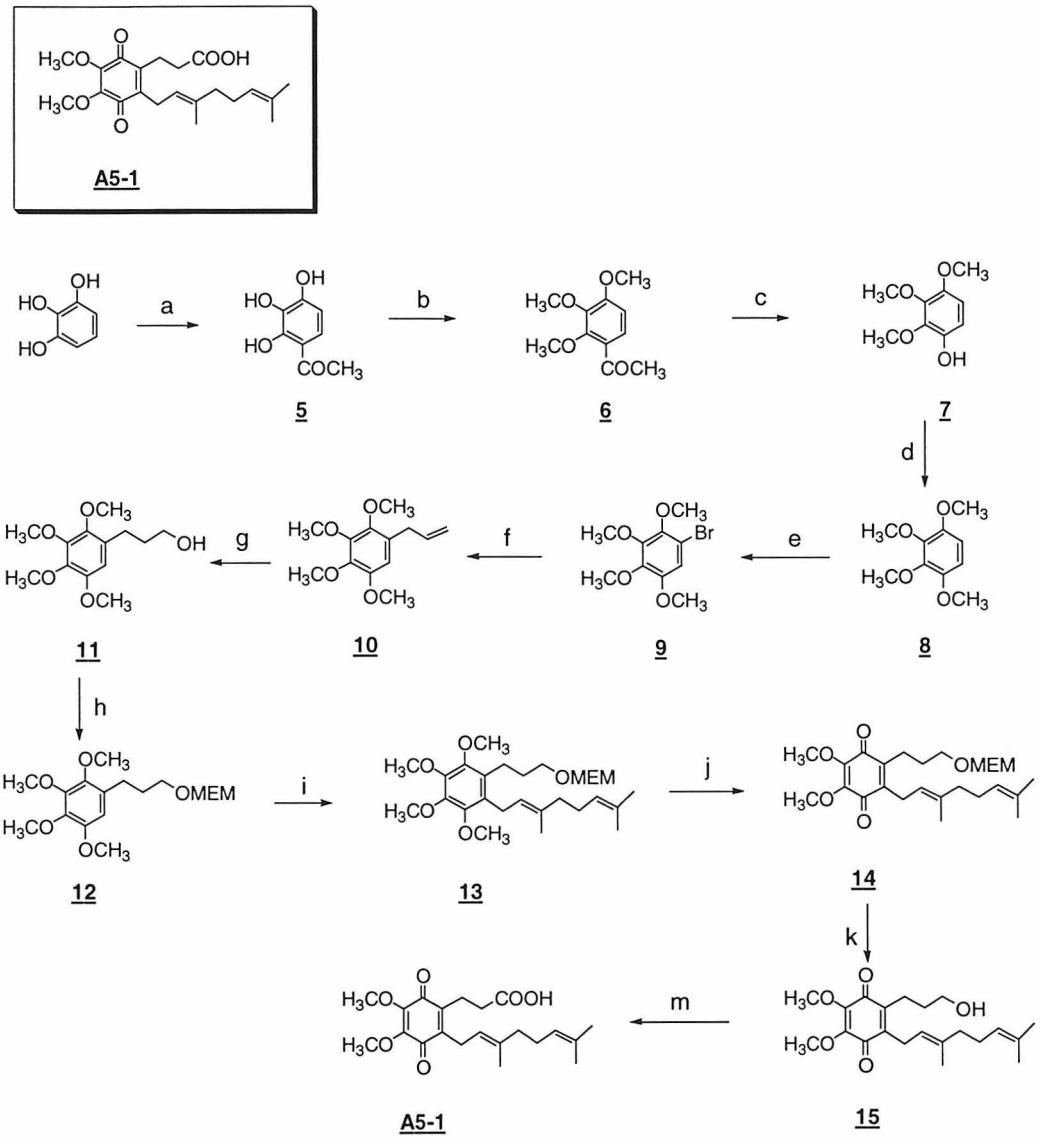
Synthesis of Ubiquinol Analogues Attached with a Carboxylic Acid Group at 5-Position. Two analogues with carboxylate functionality attached at 5-position, **A5-1** and **A5-2**, were targeted. The linker-length in both of **A5-1** and **A5-2** is two carbons. In **A5-1**, the hydrophobic tail is two isoprene units while in **A5-2** it is a saturated $C_{10}H_{21}$- alkyl chain.

Many attempts have been made to synthesize **A5-1.** The first approach is illustrated in Scheme 5.4. However, it was found that no proper hydroxy protecting group could survive the extremely acidic condition during the HBr-generating bromination reaction. A different approach as illustrated in Scheme 5.5 was pursued instead. The synthesis started with pyrogallol. In four steps, the tetramethoxybenzene **8** was obtained in a few grams quantity. The tetramethoxybenzene **8** was treated with bromine to yield a bromide, through which allyl was attached. Hydroboration-oxidation of double bond yielded the terminal alcohol, which was protected for the coupling reaction between the geranyl bromide and the highly substituted benzene. The geranylation reaction[5] was

performed with direct metalation of the ring carbon of **12** with hexane, tetramethylethylenediamine and *n*-butyllithium, followed by copper(I) catalyzed nucleophilic aromatic sustitution. The resultant **14** was oxidized with ceric ammonium nitrate (CAN) [11] and deprotected to give the alcohol **15**, which was oxidized with PDS to yield the product.



Scheme 5.4. Failed Synthesis of Ubiquinol Analogue with a Carboxylic Acid Attached to 5-Position



a) $(CH_3CO)_2O$, $ZnCl_2$; b) K_2CO_3, dimethyl sulfate; c) H_2O_2, trifluoroacetic anhydride;

d) K_2CO_3, dimethyl sulfate; e) Br_2, CH_2Cl_2; f) n-BuLi, Et_2O, CuCl, Ally bromide; g) BH_3-SMe_2, H_2O_2

h) MEMCl, diisopropylethylamine; i) n-BuLi, TMEDA, CuCl, Geranyl bromide

j) CAN, pyridine dicarboxylate; k) $ZnBr_2$; m) PDS, DMF

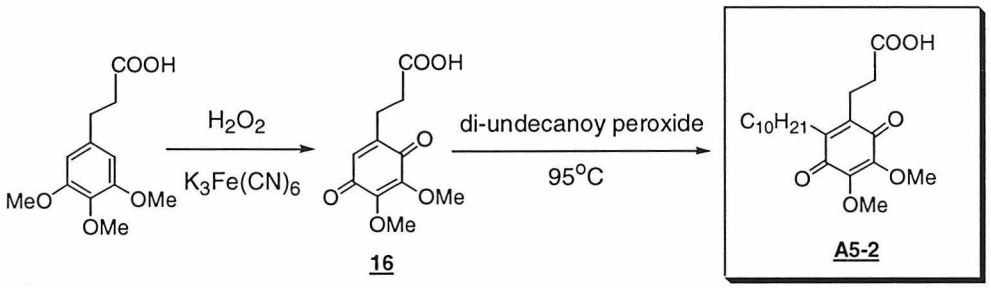
Scheme 5.5. Synthesis of Ubiquinone Analogue A5-1

The terminal hydroxy group in compound **11** is a key functionality, because the terminal hydroxy group can be activated by converting to halide or tosylate, through which the number of carbons between carboxylate and benzoquinone can be expanded by C-C bond formation coupling reactions. However, the above synthesis suffered from rather low yield, particularly in the hydroboration-oxidation step and geranylation step (yield in both reactions were less than 40%). In hydroboration-oxidation, the low yield of product might be due to the side reaction of the oxidation of electron-rich benzene ring by hydrogen peroxide in basic aqueous solution. The low yield in geranylation might be due to the steric hindrance of the highly substituted benzene ring. In addition, the overall synthesis by this scheme was rather elaborate.

In principle, analogue **A5-2** can be prepared similarly via the above procedure. However, it was found that the coupling between aromatic bromide and alkylbromide was not very efficient. Some other synthetic approaches were attempted. However, none of them were efficient and simple enough. Eventually, a two-step synthetic approach described in Scheme 5.6 was developed and afforded **A5-2** in ~20% overall yield. Although it was not a very impressive synthetic yield, it was preferred to other methods due to its consistency and easy manipulation.

The key step in this synthesis is the oxidation of the substituted benzene. In oxidative generation of benzoquinone, a well-established synthesis is the CAN oxidation of *p*-di-methoxylbenzene, which often gives a yield at >70%[11]. However, if the two methoxy groups are not at *para*-positions, the oxidation will give a very low yield or no quinone product at all. The starting material employed herein was 3-(3,4,5-trimethoxyphenyl)propionic acid, whose oxidation by CAN did not yield the desired

product at all. Matsumoto[7] et al. reported that the oxidation of 3,4,5-trimethoxytoluene with hydrogen peroxide and catalytic amount of $K_3Fe(CN)_6$ led to benzoquinone in c.a. 40% yield. This procedure was employed in the preparation of **16** and was found to yield the quinone product in 30-40% yield. The obtained **16** was then converted to ubiquinone **A5-2** via the radical coupling reaction.



Scheme 5.6. Synthesis of Ubiquinone Analogue A5-2

Attempts have also been made to synthesize another analogue as shown in Figure 5.2 through the same procedure used in the synthesis of **A5-2**. It is analoguous to **A5-2** but with only one carbon between benzoquinone and carboxylate. It was observed that the oxidation of 3,4,5-trimethoxyphenyl acetic acid gave a very low yield and the quinone product continued to decompose even after it was purified. When the coupling reaction was performed right after the benzoquinone was purified, it resulted in messy mixture after 24 hours' reaction. Therefore, no product purification was attempted further. On the other hand, it is contemplated that such an analogue may not be a good

substrate candidate since this structure might result in different redox potentials from decylubiquinone. The carboxylate is so strong an electron-withdrawing group that the substituent may change the electronic configuration of the decylbenzoquinone ring.

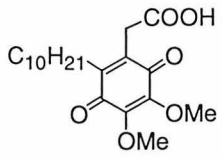
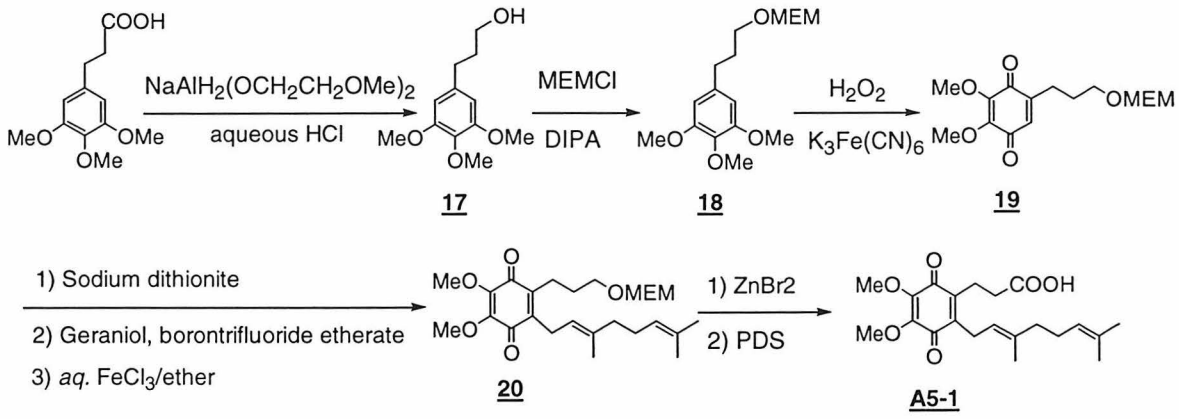


Figure 5.2. 5-Position Ubiquinol Analogue with One-carbon Linker

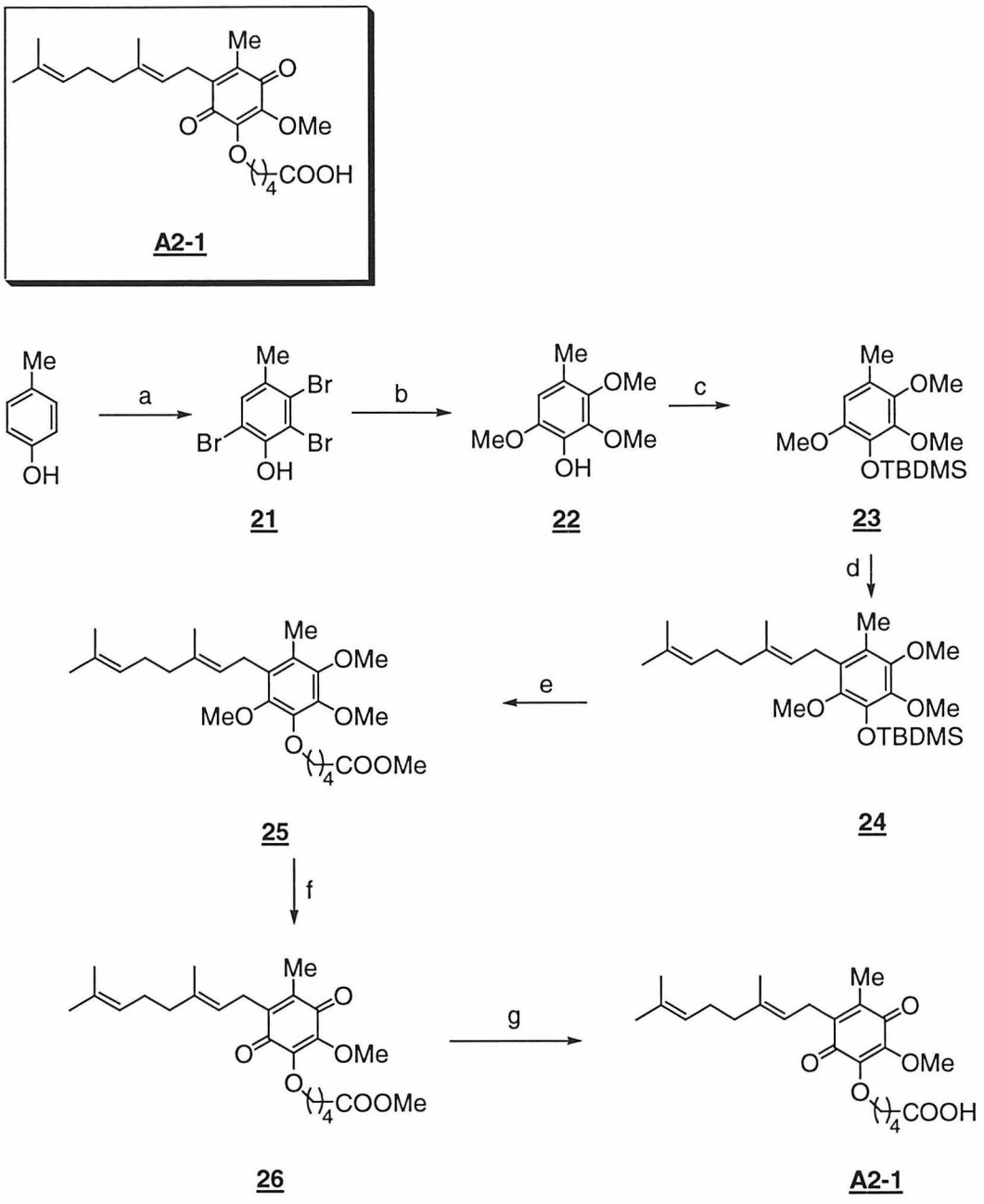
Synthesis of **A5-2** can be modified to prepare **A5-1** as shown in Scheme 5.7. The attachment of isoprene unit to the aromatic ring was achieved with the reagent of borontrifluoride etherate. Compared with other approaches, this synthesis strategy requires fewer steps and afforded to higher overall yield.



Scheme 5.7. Synthesis of Analogue A5-1

Synthesis of Ubiquinol Analogues Attached with a Carboxylic Acid Group at 2-Position or 3-Position. A series of analogues with a carboxylic acid group attached at 2- or 3-position were synthesized. The hydrophobic tails in these analogues are $C_{10}H_{21}$- for analogues **A2-2** and **A23** and isoprenoid for analogues **A2-1**. While in analogues **A2-1** and **A2-2**, the carboxylic acid group is attached selectively at position 2, analogue **A23** is a mixture of two isomeric analogues in which the carboxylic acid group was attached to position 2 and position 3 respectively.

The same synthetic strategy was followed in the preparations of **A2-1** and **A2-2**: Cresol was treated with bromine to afford tribromocresol which was then converted to trimethoxycresol via copper(I) - catalyzed nucleophilic aromatic substitution of bromide by sodium methoxide[1, 6]. In order to introduce isoprenoid units onto the benzoquinone ring, hydroxy group on the cresol was protected with silylether first. Direct metalation of the benzene ring was achieved with n-butyllithium and tetramethylethylenediamine (TMEDA) in the solvent of hexane. The geranylation reaction was performed with copper (I) catalyst and geranylbromide to yield the substituted benzene precursor to the benzoquinone. The attachment of bromoalkyl at 2-position was achieved with strong non-nucleophilic base, such as Cs_2CO_3, in the presence of poly(ethylene glycol) as a phase transfer reagent. Phenoxide anion was generated by dropwise treatment of anhydrous tetrabutylammonium fluoride (TBAF). The reaction performed under such conditions resulted in almost quantitative yield of targeted products. The CAN oxidation was performed in an ice bath. Although the standard procedure for CAN oxidation was reported to require extra 30 minutes at room temperature, it was found here that reaction at $0^{\circ}C$ for 10-30 minutes was enough to convert all the starting materials to product.

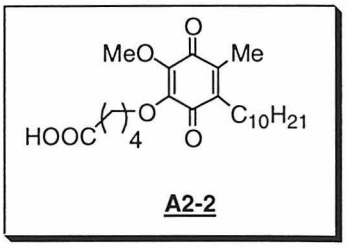


a) Br_2, Fe powder; b) MeONa, Me_2CO_3, CuCl; c) TBDMSCl, TEA, DMAP;

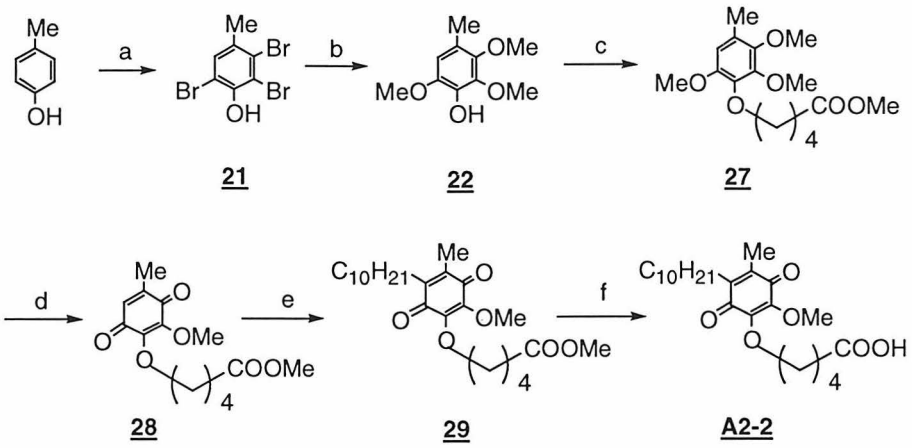
d) n-BuLi, TMEDA, CuBr, Geranyl bromide; e) TBAF, PEG, RBr,Cs_2CO_3;

f) CAN, pyridine dicarboxylate; g) LiI, pyridine

Scheme 5. 8. Synthesis of Ubiquinone Analogue A2-1

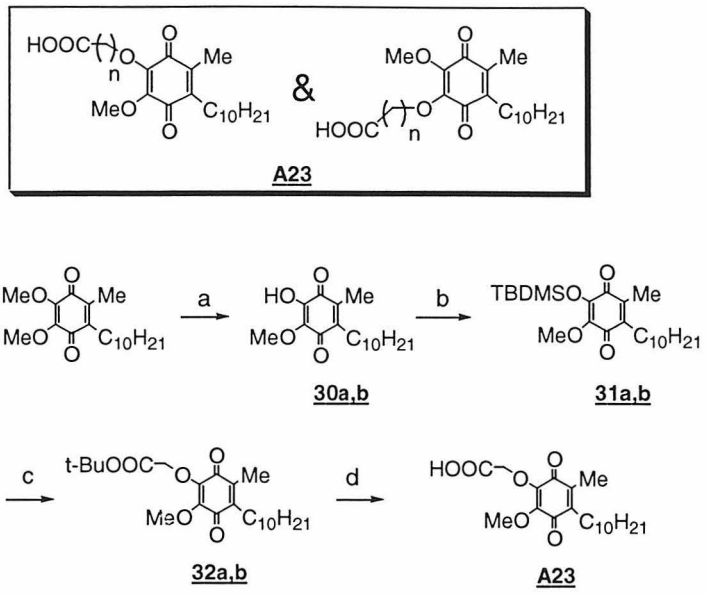


A2-2



a) Br_2, Fe powder; b) MeONa, Me_2CO_3, CuCl; c) RBr, PEG, CS_2CO_3;
d) CAN, Pyridine dicarboxylate; e) Di-undecanoy peroxide, heating;
f) LiI, pyridine, reflux

Scheme 5.9. Synthesis of Ubiquinone Analogue A2-2



a) $AlCl_3$, EtSH, -42°C; b) TBDMSCl, TEA; c) RBr, KF-Alumina; d) TFA

Scheme 5.10. Synthesis of Ubiquinone Analogue A23

Synthesis of analogue **A23** started with decyubiquinone, which can be readily prepared in several grams using the radical coupling reaction. To modify the methoxy substituent, one of the two methoxy groups on the decyubiquinone was cleaved via the treatment of ethanethiol and $AlCl_3$ at -70 °C – -42°C for 3-8 minutes. The monohydroxy decyubiquinone can be obtained at >80% and then was treated with the *tert*-butyl dimethylsilyl chloride (TBDMSCl) to protect the hydroxy group. If needed, the two isomers can be separated using silica gel flash column in this step. *Tert*-butyl bromoacetate and TBAF were used to attach the *tert*-butyl acetate onto the benzoquinone ring. An overnight reaction gave a satisfactory yield. At the last step, *tert*-butyl was removed to afford the carboxylic acid group.

Preliminary Enzymatic Activity Assays of the Ubiquinol Analogues. As a proof of concept, some of the synthesized analogues were used in enzymatic assays to screen for their bioactivities with Cytochrome bc_1. The assays were performed under enzymatic turnover conditions. As a standard procedure, 10 μM of Cytochrome c, 200 μM of ubiquinol analogues and 10 nM of Cytochrome bc_1 were used. The absorption change at the soxlet band of Cytochrome c was monitored with UV-Vis spectrometer. Figure 5.3 showed the result of this preliminary assay. The top curve is the reduction kinetics of native substrate ubiquinol-2. Although compared with ubiquinol-2, the assayed analogues are not as active as the native substrate, with analogue A5-2 and A6-1, the reduction of Cytochrome c showed encouraging kinetics behavior, which indicated that these analogues are considerably biologically active.

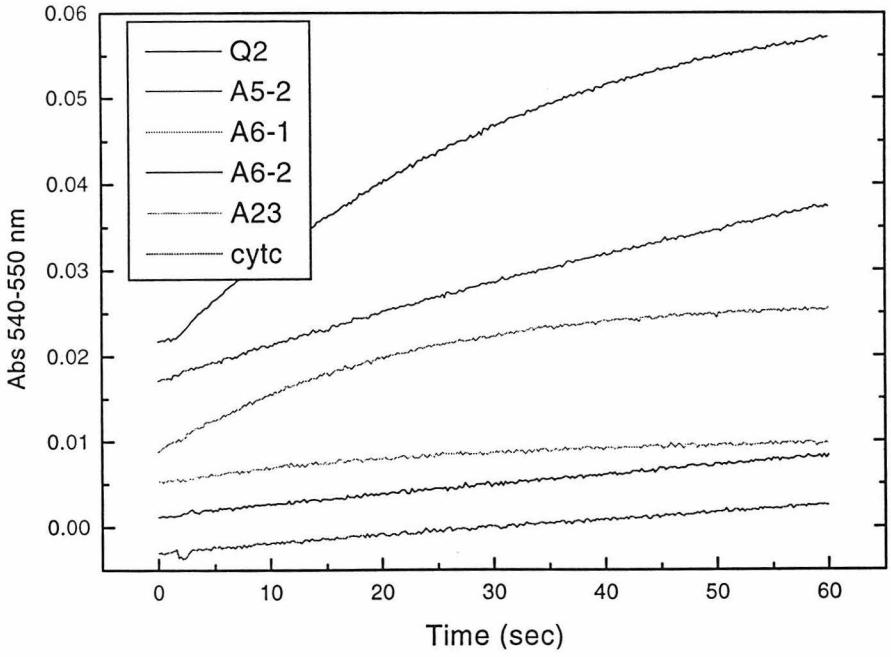
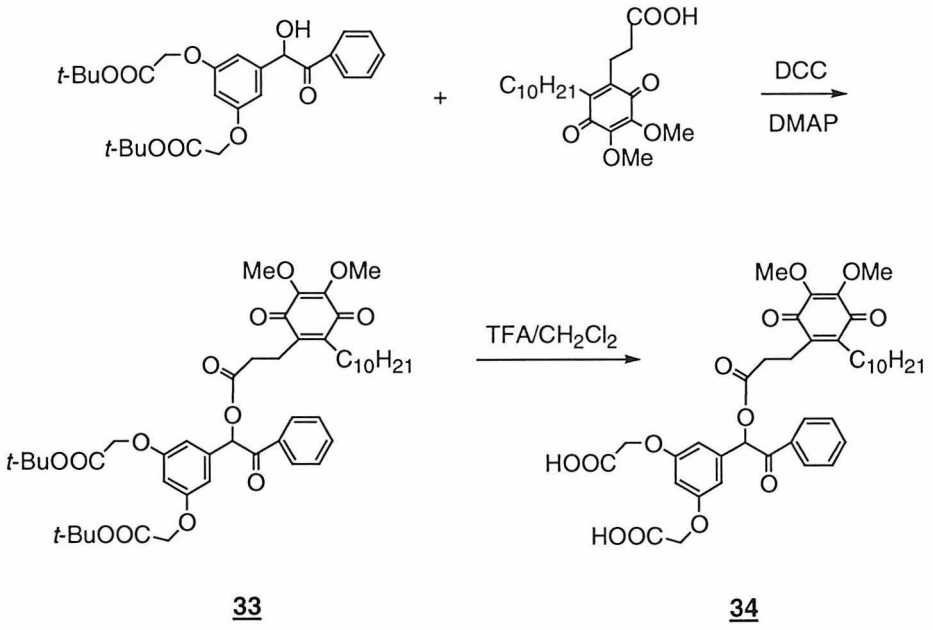


Figure 5.3. Kinetics of Cytochrome bc1 Catalyzed Cytochrome c Reduction with Native Substrate and its Analogues

Synthesis of the BCMB Caged Ubiquinone Analogue A5-2. To establish a synthetic protocol to cage the biologically active ubiquinone analogues, BCMB caged analogue A5-2 was synthesized. The carboxylic acid group on analogue A5-2 was attached to the caging group BCMB under the action of DCC and DMAP. The same reaction scheme can also be applied to other biologically active analogues. The caged ubiquinone analogue can be reduced under standard ubiquinone reducing conditions to yield caged ubiquinol analogue. From the discussions in chapter 4, it is expected that such a caged compound would release ubiquinol analogue in sub-microsecond time scale.

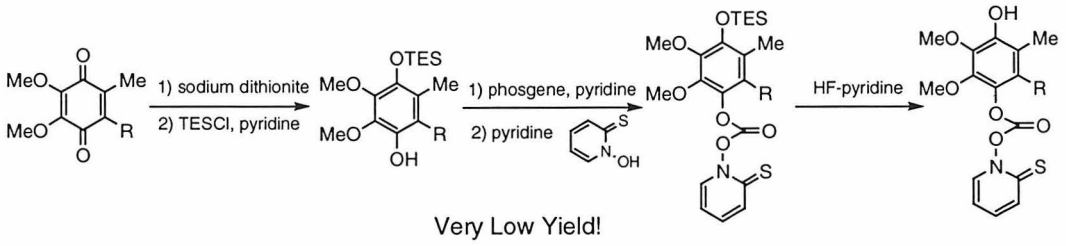


33 **34**

Scheme 5.11. Synthesis of BCMB Caged Ubiquinone Analogue A5-2

Synthesis of N-hydroxy-2-thiopyridone Caged Ubiquinol

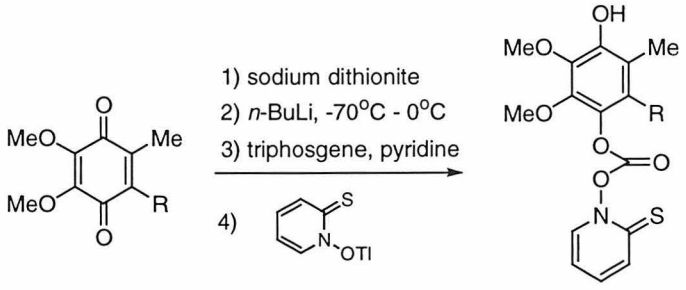
Initially, N-hydroxy-2-thiopyridone caged ubiquinol was synthesized via a three-step procedure: ubiquinone was reduced and one hydroxyl group was protected with triethylsilyl ether. The protected ubiquinol was treated with pyridine and phosgene (or triphosgene) to form the chloroformate. Without purification, it reacted with N-hydroxy-2-thiopyridone in the presence of pyridine to afford the caged ubiquinol-TES. The triethylsilyl was removed with HF-pyridine in acetonitrile at room temperature. However, this synthesis is very time-consuming and the overall is very low.



Scheme 5.12. Synthesis of N-hydroxy-2-thiopyridone Caged Ubiquinol

To improve the synthesis yield, N-hydroxy-2-thiopyridone was pre-treated with thallium ethoxide in anhydrous diethyl ether before it was added into the quinol chloroformate. Thus, coupling between N-hydroxy-2-thiopyridone and quinol chloroformate will be driven by thallium chloride precipitation. In this scheme, reaction workup and purification were much simplified. However, another two steps, the triethylsilyl protection and the silyl ether cleavge, need improvement for higher yields.

The final synthetic protocol developed was a single step one-pot reaction, in which ubiquinol was used directly to react with one equivalent of n-butyllithium at – 70°C. After slowly warming up to 0°C, the solution was equilibrated to ensure that only one hydroxyl group in each ubiquinol molecule was deprotonated. One-third equivalent of triphosgene was added with one equivalent pyridine to form the quinol chloroformate after stirring for several hours. At last, thallium salt of N-hydroxy-2-thiopyridone was added to form the targeted compound at more than 70% yield.



Scheme 5.13. Improved Synthesis of N-hydroxy-2-thiopyridone Caged Ubiquinol

Water-solubility of the synthesized N-hydroxy-2-thiopyridone caged ubiquinol-2 (HT-Q_2) was evaluated. A 50 μM solution of HT-Q_2 could be made up in a 0.1mg/ml egg lecithin solution. Preliminary photolysis study was performed in acetonitrile. Photolytic reactions of HT-Q_2 in the absence and in the presence of hydrogen donors (tributyltin hydride or mercaptoethanol) in acetonitrile indicated that the hydrogen donors accelerate the decay of semiubiquinone and lead to ubiquinol generation by competing with the disproportionation of the semiubiquinone. Its photolysis property in detergent solutions is being investigated by other members in the group.

Materials and Methods

General: 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. ^{1}H NMR spectra were recorded on a GE QE300 spectrometer operating at a nominal frequency of 300 MHz. The optical absorption was monitored with a HP 8452 diode array spectrophotometer. HPLC analysis was performed using a Shimadzu LC-6A system equipped with a Vydac C-18 reverse-phase column.

*Synthesis of **1***: 11-bromoundecanoic acid (5g, 19mmol) was dissolved in 20mL $SOCl_2$. The solution was rigorously stirred at 80-90°C for one hour. The excessive thionyl chloride was removed under reduced pressure with an aqueous trap. After removal of the excess $SOCl_2$, the resultant acyl chloride was dissolved in 30mL diethyl ether at 0°C in an ice bath and 1.2mL of 70% H_2O_2 was added. The mixture was stirred at 0°C while 1.7mL of pyridine was added dropwise over one-hour period. The ice bath was removed and the mixture was stirred at room temperature for another one hour before it was diluted with 200mL diethyl ether. The solution was washed with water, 1N HCl, water, 0.5 M $NaHCO_3$ and water again sequentially and then dried over anhydrous sodium sulfate. 4.7g (8.8mmol) bromoundecanoyl peroxide was obtained upon the removal solvent. ^{1}H NMR was used to confirm there was not any pyridine impurity left in the crude mixture.

A mixture of 2,3-dimethoxy-5-methyl benzoquinone (Q_0) 0.45g (3mmol) and 1.5g of bromoundecanoyl peroxide in 20mL of glacial acetic acid was heated at 95°C for 14

hours. To the mixture was added heptane to remove the acetic acid *in vacuo*. The resultant slurry was redissolved in diethyl ether and washed with 0.5M $NaHCO_3$ and water. The ether solution was dried over magnesium sulfate. After removal of the solvent, the residue was separated with silica gel flash column with 20/1 hexanes and ether. Yield: 0.51g (43% based on Q_0). ^1H NMR ($CDCl_3$) δ 3.98 (s, 6H), 3.00 (t, 2H), 2.48 (t, 2H), 2.02 (s, 3H), 1.3 (m, 16H). MS (FAB) m/z calcd 401.335, obsd 401.337.

Synthesis of ***2***: 16-Bromohexadecanoic acid (5g, 15mmol) was used as the starting material. The synthesis followed the same procedure as in the synthesis of **1.** The crude yield of the bromohexadecanoyl peroxide was 4.7g (6.9mmol). 2,3-dimethoxy-5-methyl benzoquinone (Q_0) 0.45g (3mmol) and 2.0g of bromohexadecanoyl peroxide were used for the coupling reaction. Yield: 0.54g (38% based on Q_0). ^1H NMR ($CDCl_3$) δ 3.98 (s, 6H), 3.01 (t, 2H), 2.48 (t, 2H), 2.02 (s, 3H), 1.3 (m, 26H). MS (FAB) m/z calcd 471.468, obsd 471.465.

Synthesis of ***3***: To a suspension of 450mg of mercuric oxide in 5mL of dimethoxyethane was added 0.35mL of 70% perchloric acid. The mixture was warmed at 60°C until the solid was dissolved and 0.4mL of water was added. Compound **1** (0.17g, 0.4mmol) was added into the clear solution and the resulting mixture was heated at 60°C for 2 hours. The solution was extracted with diethyl ether and the organic phase was washed with sodium bicarbonate and water, dried with magnesium sulfate and concentrated. The crude product was purified on silica gel flash column with 2% ethanol in 4/1 hexane and ethyl acetate solvent. Yield 0.12g (87%), ^1H NMR ($CDCl_3$) δ 3.99 (s, 6H), 3.63 (t, 2H), 2.8 (br

s, 1H), 2.4 (m, 2H), 2.02 (s, 3H), 1.32 (m, 16H). MS (FAB) m/z (MH^+) calcd 339.446, obsd 339.443.

*Synthesis of **4***: Synthesis of **4** followed the same procedure as described in the synthesis of **3**. Yield 94%. ^1H NMR ($CDCl_3$) δ 3.98 (s, 6H), 3.63 (t, 2H), 2.8 (br s, 1H), 2.4 (m, 2H), 2.02 (s, 3H), 1.32 (m, 26H). MS (FAB) m/z (MH^+) calcd 409.579, obsd 409.578.

*Synthesis of **A6-1***: Pyridium dichromate (PDC) (0.28g, 0.75mmol) was dissolved in 5mL DMF and cooled to 0°C in an ice bath. Compound **3** (0.1g, 0.3mmol) was added into the solution and stirred for 4 hours. 20mL 0.01N HCl aqueous solution was added and the product was extracted quickly with diethyl ether. The organic solution was concentrated and loaded to silica gel flash column, eluted with 2% acetic acid in 2/1 hexanes-ethylacetate solution. Yield 92mg (87%). ^1H NMR ($CDCl_3$) δ 3.98 (s, 6H), 2.45 (t, 2H), 2.37 (t, 2H), 2.00 (s, 3H), 1.61 (bs, 2H), 1.30 (m, 12H).

*Synthesis of **A6-2***: The same procedure in the synthesis of **A6-1** was followed. Yield 84%. ^1H NMR ($CDCl_3$) δ 3.99 (s, 6H), 3.50 (t, 2H), 2.35 (t, 2H), 2.00 (s, 3H), 1.74 (bs, 2H), 1.25 (m, 22H).

*Synthesis of **5***: In a round-bottomed flask, 7g (52.5 mmol) of fine powder zinc chloride is dissolved in 10mL of glacial acetic acid by heating at 135-140°C. Acetic anhydride 10g was added to the clear, pale brown solution, followed by the addition of 12.5g (0.1 mol) of pyrogallol. The mixture was refluxed for an hour under rigorous stirring. The excess

acetic acid and acetic anhydride was removed under reduced pressure. Water was added and the solution was stirred for 10 minutes and then cooled in ice bath, filtered with suction and washed with cold water. The crude product was crystallized from boiling water saturated with sulfur dioxide and the needle-like crystal was collected. By saturating the mother liquor with salt and cooling to 0°C, more crystal was obtained. The two batches of crystal collected were combined and purified by recrystallization to yield pure product. Yield 9.6g (57%). ^{1}H NMR ($CDCl_3$) δ 7.28 (d, 1H), 6.71 (d, 1H), 2.32 (s, 3H).

Synthesis of ***6***: A mixture of **5** (9.2g, 55mmol), K_2CO_3 (44g, 0.32mol), dimethyl sulfate (16mL, 0.17mol) and 100mL acetone was refluxed for 24 hours. Acetone was removed under reduced pressure and water was added. The mixture was extracted with diethyl ether and the organic phase was concentrated. The product was purified with vacuum distillation to yield 10.3g. Yield 90.5%. ^{1}H NMR ($CDCl_3$) δ 7.23 (d, 1H), 6.46 (d, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 3.69 (s, 3H), 2.44 (s, 3H).

Synthesis of ***7***: To a stirred solution of 100mL CH_2Cl_2 and 70% hydrogen peroxide (2.7g, 59mmol), trifluoroacetic anhydride (8g, 38mmol) was added dropwise at –30 to -25°C. The mixture was stirred for 20 minutes at -30°C after the addition and the temperature was lowered to -40°C. A solution of **6** (6.3g, 30mmol) in CH_2Cl_2 was added dropwise under nitrogen flow. The mixture was stirred at -40°C for an additional 20 minutes and allowed to warm up to room temperature. Washed with sodium bicarbonate aqueous solution and water alone, dried with sodium magnesium, the organic solution was

concentrated *in vacuo* to give an oily residue. To the resultant oil, a solution of potassium hydroxide (4g) in 30mL methanol was added and the mixture was refluxed for 30 minutes. Water was added and the aqueous phase was washed with diethyl ether. The resultant aqueous phase was acidified with 0.1N HCl solution and saturated with salt and extracted with diethyl ether. The ether solution was concentrated and the product was purified by vacuum distillation. Yield 4.3g (78%). ^{1}H NMR ($CDCl_3$) δ 6.40 (d, 1H), 6.11 (d, 1H), 6.02 (bs, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H).

Synthesis of ***8***: A mixture of **7** (7g, 38mmol), dimethyl sulphate (4mL, 42mmol), K_2CO_3 (14g, 100mmol) and acetone 100mL was refluxed for 22 hours. The solvent was removed under reduced pressure and water was added to the residue. After several hours at room temperature, crystals formed and were collected, washed with water and dried. Upon recrystallization in methanol, the product was obtained. Yield 7.3g (97%). ^{1}H NMR ($CDCl_3$) δ 6.15 (d, 2H), 3.84 (s, 6H), 3.82 (s, 6H). MS (FAB) m/z (MH^+) calcd. 198.2158, obsd. 198.2157.

Synthesis of ***9***: To a cold solution of **8** (3.96g, 20mmol) and 5mg of iron power in chloroform (50mL), Br_2 (3.3g, 20mmol) in 10mL was added dropwise at 0°C. A base trap was connected with the reaction flask to release the HBr pressure. After 3 hours, the solution was successively washed with water, diluted sodium hydroxide and dried over anhydrous sodium sulfate. The product was purified via vacuum distillation. Yield 4.88g (88%). ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 1H), 4.14 (s, 3H), 4.09 (s, 9H). The bromination was also confirmed by GC-MS.

*Synthesis of **10***: To a stirred and ice cooled diethyl ether solution of **9** (2.77g, 10mmol), *n*-Butyllithium (4.2mL, 10.5mmol) in hexanes solution was added dropwise under argon flow. During the addition, the formation a white precipitate was observed. The mixture was stirred for 30 minutes at 0°C and copper (I) bromide (2g, 7mmol) was added in portions. The mixture became homogeneous while stirred for 2 hours. The allyl bromide (1.3g, 10.7mmol) dissolved in 10mL diethyl ether was added dropwise. The mixture was stirred for 2 hours at 0°C and another 4 hours at room temperature. It was quenched with diluted HCl solution and extracted with diethyl ether. The ether solution was dried over magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography using hexanes as eluent. Yield 1.81g (76%). ^{1}H NMR ($CDCl_3$) δ 6.27 (s, 1H), 6.00 (m, 1H), 5.40-5.07 (m, 2H), 4.25 (s, 3H), 4.14 (s, 3H), 4.10 (s, 3H), 4.07 (s, 3H), 3.70 (d, 2H).

*Synthesis of **11***: A flame dried round-bottom flask was charged with a stir bar, **10** (3.57g, 15mmol) and anhydrous hexane (30mL). The solution was cooled to 0°C in an ice bath. Borane-methyl sulfide (5. 1mmol) was added dropwise and then the solution was allowed to warm up to room temperature and stirred for another 1 hr. Ethanol (50mL) was then added followed by 50mL 3N sodium hydroxide solution. After cooled back to 0°C again, hydrogen peroxide (2mL of 30% solution) was added slowly. Ice bath was removed and the reaction mixture was stirred for another 4 hr under heating. The mixture was then poured into ice water, extracted with ether. The organic extract was dried over anhydrous magnesium sulfate, concentrated under reduced pressure. Silica gel flash column chromatography purification (1% ethanol 4/1 hexanes/ethyl acetate eluent) yielded **11**

2.6g (68%) yield. ^{1}H NMR ($CDCl_3$) δ 6.23 (s, 1H), 5.15 (bs, 1H), 3.90 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.74 (s, 3H), 3.59 (t, 2H), 2.62 (t, 2H), 1.85 (m, 2H).

*Synthesis of **12***: Compound **11** (1.28g, 5mmol) was dissolved in 25mL methylene dichloride. To the solution, MEM chloride (0.75g, 6mmol) was added followed by *N, N*-diisopropylethylamine (0.78g, 6mmol). The resultant solution was stirred for 24 hours at room temperature, quenched by ammonium chloride aqueous solution and extracted with diethyl ether. The ether phase was collected, dried over magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography using 4/1 hexanes/ethyl acetate as eluent. Yield 1.62g (94%). ^{1}H NMR ($CDCl_3$) δ 6.23 (s, 1H), 4.56 (s, 2H), 3.90 (s, 3H), 3.78 (s, 3H), 3.77 (t, 2H), 3.76 (s, 3H), 3.74 (s, 3H), 3.63 (m, 4H), 3.37 (s, 3H), 2.62 (t, 2H), 1.87 (m, 2H).

*Synthesis of **13***: *n*-Butyllithium (1.4mL of 2.5M solution in hexanes) was added over 30 minutes under argon to a solution of **12** (0.8g, 2.3mmol) in 10mL hexane containing tetrmethylethylenediamine (TMEDA) (0.5g) at 0°C. The mixture was stirred for an additional 30 minutes, followed by addition of THF (25mL) and CuCN (0.3g, 3.3mmol). After the mixture was stirred for 30 minutes, a solution of geranyl bromide (0.5g, 2.3mmol) in THF (10mL) was added slowly over 60 minutes, and the mixture was stirred for additional 60 minutes. The reaction was then quenched with saturated aqueous ammonium chloride. The mixture was extracted with diethyl ether and the organic phase was separated, washed with aqueous NH_4OH, water and brine and dried over magnesium sulfate. The solvent was removed under reduced pressure and the residue was separated

by silica gel flash column chromatography using 20/1 hexanes - ethylacetate eluent to yield a colorless oil. Yield 0.52g (47%). ^{1}H NMR ($CDCl_3$) δ 5.08(t, 1H), 4.96 (t, 1H), 4.56 (s, 2H), 3.79 (ds, 6H), 3.73 (ds, 6H), 3.63 (m, 4H), 3.59 (t, 2H), 3.37 (s, 3H), 3.30 (d, 2H), 2.68 (t, 2H), 2.05-1.92 (m, 4H), 1.79 (m, 2H), 1.69 (s, 3H), 1.64 (s, 3H), 1.59(s, 3H).

*Synthesis of **14***: Pyridine-2, 6-dicarboxylate (0.42g, 2.5mmol) was added to a cold (0°C) solution of **13** (0.48g, 1mmol) in 20mL of 7:3 acetonitrile/water. An ice-cold solution of ceric ammonium nitrate (CAN) (1.37g, 2.5mmol) in 20mL of 1:1 acetonitrile/water was slowly added over 20 minutes. The mixture was stirred at 0°C for 20 minutes and the ice bath was removed. TLC was employed to monitor the reaction. Normally within 10 minutes, the starting material would disappear. The mixture was poured into 40mL of water and extracted with methylene chloride. The organic layers collected were dried over $MgSO_4$, followed by solvent removal under reduced pressure and silica gel flash column chromatography (20:1 hexanes/ether). Yield 0.36g (81%). ^{1}H NMR ($CDCl_3$) δ 5.02 (t, 1H), 4.91 (t, 1H), 4.84 (s, 2H), 3.99 (s, 3H), 3.98 (s, 3H), 3.63 (m, 4H), 3.42 (t, 2H), 3.37 (s, 3H), 3.17 (d, 2H), 2.45 (t, 2H), 2.07 (m, 4H), 1.719 (s, 3H), 1.649 (s, 3H), 1.573 (s, 3H), 1.52 (m, 2H),

*Synthesis of **15***: Compound **14** (0.27g, 0.6mmol) was dissolved in 20mL anhydrous methylene dichloride and cooled to 0°C in ice bath. Under argon flow, anhydrous zinc bromide (0.68g, 3mmol) was added into the solution. TLC was used to check the progress of the reaction. When the starting material disappeared, NH_4OH solution was

added and the mixture was extracted with diethyl ether. The organic phase was collected, dried over anhydrous sodium sulfate and concentrated. The product was purified with silica gel flash column chromatography (2% ethanol in 4/1 hexanes - ethyl acetate). Yield 0.19g (87%). ^{1}H NMR ($CDCl_3$) δ 5.03 (t, 1H), 4.91(t, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.58 (t, 2H), 3.16(d, 2H), 2.30 (t, 2H), 2.07 (m, 4H), 1.79 (m, 2H), 1.721 (s, 3H), 1.648 (s, 3H), 1.58 (s, 3H).

*Synthesis of **A5-1***: Pyridium dichromate (PDC) (0.21g, 0.55mmol) was dissolved in 5mL DMF and cooled to 0°C in an ice bath. Compound **15** (0.1g, 0.27mmol) was added into the solution. TLC was used to monitor the reaction. 10mL water was added and the product was extracted quickly with diethyl ether. The organic solution was concentrated and loaded to silica gel flash column, eluted with 1% acetic acid in 4/1 hexanes-ethylacetate solution. Yield 85mg (84%). ^{1}H NMR ($CDCl_3$) δ 5.03 (t, 1H), 4.94 (t, 1H), 3.18 (d, 2H), 3.98 (s, 3H), 3.99 (s, 3H), 2.89 (m, 2H), 2.97 (m, 2H), 2.10 (m, 4H), 1.72 (s, 3H), 1.64 (s, 3H), 1.57 (s, 3H).

*Synthesis of **16***: To a mixture of 3-(3,4,5-trimethoxyphenyl) propionic acid (3g, 12.5mmol), acetonitrile (12g) and water (1.2g), 100mg of $K_6[Fe(CN)_6]$ was added. To the mixture was added 30% hydrogen peroxide solution (3g) and stirred for 48 hour. The color of the solution became dark red and TLC was used to monitor the reaction. The mixture was acidified with 0.01N HCl solution, extracted with diethyl ether. The organic layers were collected, dried over magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography using 2% acetic acid in 2:1

hexanes/ethyl acetate. Yield 1.1g (37%). ^{1}H NMR ($CDCl_3$) δ 5.301 (s, 1H), 4.024 (s, 3H), 4.003 (s, 3H), 2.755 (t, 2H), 2.627 (t, 2H).

*Synthesis of **A5-2***: Undecanoic acid (12.7g, 68mmol) was dissolved in 20mL $SOCl_2$. The solution was rigorously stirred at 80-90°C for one hour. The excessive thionyl chloride was removed under reduced pressure with an aqueous trap. The resultant acyl chloride was dissolved in 120mL diethyl ether at 0°C in an ice bath and 4mL of 70% H_2O_2 was added. The mixture was stirred at 0°C while 6mL of pyridine was added dropwise over one-hour period. The ice bath was removed and the mixture was stirred at room temperature for another one hour before it was diluted with 700mL diethyl ether. The solution was washed with water, 1N HCl, water, 0.5 M $NaHCO_3$ and water again sequentially and then dried over anhydrous sodium sulfate. 11.5g (31mmol) undecanoyl peroxide was obtained upon the removal solvent. ^{1}H NMR was used to confirm there was not any pyridine impurity left in the crude mixture.

A mixture of **16** (0.72g, 3mmol) and 1.8g of undecanoyl peroxide in 20mL of glacial acetic acid was heated at 95°C for 14 hours. Heptane was added to the mixture and the acetic acid was removed *in vacuo*. The resultant slurry was re-dissolved in diethyl ether and washed with 0.5M $NaHCO_3$ and water. The ether solution was dried over magnesium sulfate. After removal of the solvent, the residue was separated with silica gel flash column using 1% acetic acid in 4:1 hexanes/ethyl acetate. Yield 457mg (40%). ^{1}H NMR ($CDCl_3$) δ 3.992 (s, 6H), 2.77 (t, 2H), 2.52 (t, 2H), 2.43 (t, 2H), 1.8-1.1 (m, 16H), 0.87 (t, 3H).

Synthesis of 17: To an ice-cold solution of 3-(3,4,5-trimethoxyphenyl) propionic acid (1g, 4 mmol) in 20 mL of THF was added 5.66 mL (20mmol) of 70% $NaAlH_2(OCH_2CH_2OCH_3)_2$ in benzene over 5 minutes. The mixture was stirred at room temperature for 1 hour and then poured into a mixture of ice and 3N HCl. The solution was extracted with diethyl ether. Organic layers collected were combined and washed with 1N hydrochloric solution and sodium bicarbonate solution. After the removal of solvent under reduced pressure, the residue was purified with silica gel flash chromatography (2% ethanol in 4:1 hexanes/ethyl acetate). Yield 850mg (90%). ^{1}H NMR ($CDCl_3$) δ 6.27 (s, 2H), 3.81 (s, 6H), 3.69 (s, 3H), 3.59 (t, 2H), 2.70 (t, 2H), 1.77 (m, 2H).

Synthesis of 18: To a methylenechloride (25mL) solution of compound **17** (1.13g, 5mmol) was added MEM chloride (0.75g, 6mmol) followed by *N, N*-diisopropylethylamine (0.78g, 6mmol). The mixture was stirred for 24 hours at room temperature, quenched by ammonium chloride aqueous solution and extracted with diethyl ether. The ether phase was collected, dried over magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography (4/1 hexanes/ethyl acetate). Yield 1.51g (96%). ^{1}H NMR ($CDCl_3$) δ 6.301 (s, 2H), 4.559 (s, 2H), 3.816 (s, 6H), 3.69 (t, 2H), 3.61 (t, 2H), 3.47 (t, 2H), 3.37 (s, 3H), 2.47 (t, 2H), 1.77 (m, 2H).

Synthesis of 19: To a mixture of **18** (0.8g, 2.5mmol), acetonitrile (2.4g) and water (0.24g), 40mg of $K_6[Fe(CN)_6]$ was added, followed by 0.6 g of 30% hydrogen peroxide solution. The color of the solution became dark red gradually and TLC was used to

monitor the reaction. The mixture was allowed to stir for 48 hour and then extracted with diethyl ether. The organic layers were collected, dried over magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography using 3:1 hexanes/ethyl acetate. Yield 276mg (35%). ^{1}H NMR ($CDCl_3$) δ 6.372 (s, 1H), 4.556 (s, 2H), 3.90 (s, 6H), 3.61 (m, 4H), 3.51 (t, 2H), 3.373 (s, 3H), 2.30 (t, 2H), 1.54 (m, 2H).

*Synthesis of **20***: To a solution of **19** (157mg, 0.5mmol) in acetonitrile (5 mL) was added sodium dithionite (0.6g) aqueous solution (5mL). The mixture was vigorously stirred under nitrogen flow until the dark red color dissipated, then was extracted with diethyl ether. The diethyl ether solution was dried over magnesium sulfate and concentrated under reduced pressure. The resultant colorless residue was dissolved in 10 mL dioxane together with geraniol (0.12g, 0.8mmol). BF_3-Et_2O (0.43g, 3mmol) was added dropwise to the solution under nitrogen flow at room temperature. The solution was allowed to stir for 60 minutes and treated with a solution of $FeCl_3$ (1g) in 80% methanol (10mL). The mixture was stirred for 10 minutes and then diluted with water and extracted with diethyl ether. The ether solution was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The product was purified by silica gel flash chromatography (10:1 hexanes/ether). Yield 101mg (45%). ^{1}H NMR ($CDCl_3$) δ 5.02 (t, 1H), 4.91 (t, 1H), 4.84 (s, 2H), 3.99 (s, 3H), 3.98 (s, 3H), 3.63 (m, 4H), 3.42 (t, 2H), 3.37 (s, 3H), 3.17 (d, 2H), 2.45 (t, 2H), 2.07 (m, 4H), 1.719 (s, 3H), 1.649 (s, 3H), 1.573 (s, 3H), 1.52 (m, 2H).

*Synthesis of **A5-1** (Scheme 5.7)*: To an ice-cold solution of **20** (45mg, 0.1mmol) in anhydrous dichloromethane (5mL) was added anhydrous $ZnBr_2$ (0.12g). The mixture was stirred vigorously under argon flow. The MEM protection group was removed in 15 minutes as indicated by TLC. NH_4OH solution was added and the mixture was extracted with diethyl ether. The organic phase was collected, dried over anhydrous sodium sulfate and concentrated. The residue was re-dissolved in 2mL DMF and was added into an ice-cold solution of pyridium dichromate (PDC) (0.12g, 0.30mmol) in 3mL DMF. The mixture was stirred for 20 minutes and 10mL water were added. The product was extracted with diethyl ether. The ether solution was concentrated and loaded to silica gel flash column, eluted with 1% acetic acid in 4/1 hexanes-ethylacetate solution. Yield 30mg (79%). ^1H NMR ($CDCl_3$) δ 5.03 (t, 1H), 4.94 (t, 1H), 3.98 (s, 3H), 3.99 (s, 3H), 3.18 (d, 2H), 2.97 (m, 2H), 2.89 (m, 2H), 2.10 (m, 4H), 1.73 (s, 3H), 1.66 (s, 3H), 1.58 (s, 3H).

*Synthesis of **21***: *p*-Cresol (21.6g, 0.2mol) was dissolved in chloroform (50mL) containing iron powder (0.8g, 14mmol). The flask was connected with an aqueous base trap to release the pressure generated in the reaction. Bromine (102.4g, 0.64mol) was then added dropwise over 5 hours at room temperature. The solution was stirred for additional 48 hours and then filtered, washed with dilute aqueous $NaHSO_3$, and dried over magnesium sulfate. The solvent was removed under reduced pressure and the residue was recrystallized from hexanes to yield the product. Yield 62.7g (91%). ^1H NMR ($CDCl_3$) δ 7.382 (s, 1H), 5.880 (s, 1H), 2.395 (s, 3H).

*Synthesis of **22***: The mixture of sodium methoxide (40mL 20% methanol solution), DME (30mL) and dimethyl carbonate (5mL) was heated under reduced pressure to remove most of the methanol. CuCl (1.7g, 16.7mmol) was introduced into the mixture. A solution of **21** (3.44g, 10.3mmol) in 10mL of DME was added dropwise over 3 hours while the temperature was kept at 80°C. The mixture was stirred at this temperature for 5 hours, quenched by water, acidified with 1N HCl solution to pH 2~3. The solid was filtered off and the liquid was extracted with diethyl ether. Organic layers were collected, combined, dried over anhydrous magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography with 4:1 hexanes/ethylacetate as eluent. Yield 3.03g (90%). ^{1}H NMR ($CDCl_3$) δ 6.430 (s, 1H), 5.440 (s, 1H), 3.934 (s, 3H), 3.841 (s, 3H), 3.781 (s, 3H), 2.200 (s, 3H).

*Synthesis of **23***: To a mixture of **22** (3.96g, 20mmol) and TBDMS chloride (3.45g, 22mmol) in 50mL THF, triethylamine (TEA) (2.3g, 23mmol) and catalytic amount of DMAP was added. The mixture was stirred for overnight, quenched with water, extracted by diethyl ether. The ether solution collected was dried over magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography using 4:1 hexanes/ethyl acetate as eluent. Yield 5.8g (93%). ^{1}H NMR ($CDCl_3$) δ 6.390 (s, 1H), 3.818 (s, 3H), 3.770 (s, 3H), 3.745 (s, 3H), 2.202 (s, 3H), 1.013 (s, 9H), 0.144 (s, 6H).

*Synthesis of **24***: *n*-Butyllithium (2.8mL of 2.5M solution in hexanes) was added over 30 minutes under argon to a solution of **19** (1.44g, 4.6mmol) in 10mL hexane containing tetrmethylethylenediamine (TMEDA) (1g) at 0°C. The mixture was stirred for an

additional 30 minutes, followed by addition of THF (40mL) and CuCN (0.6g, 6.6mmol). After the mixture was stirred for 30 minutes, a solution of geranyl bromide (1g, 4.6mmol) in THF (15mL) was added slowly over 60 minutes, and the mixture was stirred for additional 60 minutes. The reaction was then quenched with saturated aqueous ammonium chloride. The mixture was extracted with diethyl ether and the organic phase was separated, washed with aqueous NH_4OH, water and brine and dried over magnesium sulfate. The solvent was removed under reduced pressure and the residue was separated by silica gel flash column chromatography using 20/1 hexanes - ethylacetate as eluent to yield a colorless oil. Yield 1.11g (54%). ^{1}H NMR ($CDCl_3$) δ 5.11 (t, 1H), 5.06 (t, 1H), 3.798 (s, 3H), 3. 741 (s, 3H), 3. 752 (s, 3H), 3.10 (d, 2H), 2.107 (s, 3H), 1.98 (m, 4H), 1.69 (s, 3H), 1.64 (s, 3H), 1.59 (s, 3H), 0.994 (s, 9H), 0.139 (s, 6H).

*Synthesis of **25***: TBAF (1.1mL of 1M solution in THF) was added 20mL anhydrous acetonitrile twice and dried with rotary evaporator twice and re-dissolved in 3mL anhydrous acetonitrile. To a mixture of **24** (0.45g, 1mmol), methyl 5-bromovalerate (0.78g, 4mmol) and PEG-2000 (80mg) in 20mL anhydrous acetonitrile, cesium carbonate (0.65g, 2mmol) and TBAF acetonitrile solution (or KF-alumina 0.3g) was added. The mixture was stirred for 12 hours under argon flow. The solid was filtered off and the liquid was concentrated. The residue was separated by silica gel flash column chromatography using 4:1 hexanes/ethylacetate as eluent. Yield 0.41g (91%). ^{1}H NMR ($CDCl_3$) δ 5.06 (t, 1H), 4.94 (t, 1H), 4.12 (t, 2H), 3.791 (s, 3H), 3.782 (s, 3H), 3.745 (s, 3H), 3.574 (s, 3H), 3.05 (d, 2H), 2.25 (t, 2H), 1.92-2.05 (m, 4H), 1.72 (s, 3H), 1.71-1.66 (m, 4H), 1.691 (s, 3H), 1.643 (s, 3H).

*Synthesis of **26***: Pyridine-2, 6-dicarboxylate (0.42g, 2.5mmol) was added to a cold (0°C) solution of **25** (0.45g, 1mmol) in 20mL of 7:3 acetonitrile/water. An ice cold solution of ceric ammonium nitrate (CAN) (1.37g, 2.5mmol) in 20mL of 1:1 acetonitrile/water was slowly added over 20 minutes. The mixture was stirred at 0°C for 20 minutes and the ice bath was removed. The progress of the reaction was monitored by TLC. Normally within 10 minutes, the starting material would disappear. The mixture was poured into 40mL of water and extracted with methylene chloride. The organic layers was collected, dried over $MgSO_4$, followed by solvent removal under reduced pressure and quickly transferred to silica gel flash column chromatography using 5:1 hexanes/ethyl acetate as eluent. Yield 0.34g (81%). ^{1}H NMR ($CDCl_3$) δ 5.07 (t, 1H), 4.86 (t, 1H), 4.13 (t, 2H), 3.901 (s, 3H), 3.571 (s, 3H), 3.16 (d, 2H), 2.25 (t, 2H), 2.07 (m, 4H), 1.934(s, 3H), 1.86 (m, 2H), 1.666 (s, 3H), 1.65 (m, 2H), 1.602 (s, 3H), 1.583 (s, 3H).

*Synthesis of **A2-1***: To a solution of **26** (0.5mmol) in 10mL pyridine was added LiI (0.14g, 1mmol). The mixture was heated at 90°C and monitored by TLC. After the starting material disappeared, the reaction was quenched with water, acidified with 0.01N HCl solution and extracted with diethyl ether. The ether solution was dried and concentrated *in vacuo*. The residue was separated with silica gel flash column chromatography using 1% acetic acid in 4:1 hexanes/ethyl acetate. Yield 87%. ^{1}H NMR ($CDCl_3$) δ 5.07 (t, 1H), 4.86 (t, 1H), 4.11 (t, 2H), 3.901 (s, 3H), 3.571 (s, 3H), 3.16 (d, 2H), 2.35 (t, 2H), 2.07 (m, 4H), 1.934(s, 3H), 1.82 (m, 2H), 1.666 (s, 3H), 1.64 (m, 2H), 1.602 (s, 3H), 1.583 (s, 3H).

Synthesis of 27: To a mixture of **22** (1.0g, 5mmol), methyl 5-bromovalerate (3.1g, 20mmol) and PEG-2000 (150mg) in 20mL anhydrous acetonitrile, cesium carbonate (3.25g, 10mmol) was added under argon flow. The mixture was stirred for 20 hours under argon atmosphere. The solid was filtered off and the liquid was concentrated. The residue was separated by silica gel flash column chromatography using 4:1 hexanes/ethylacetate as eluent. Yield 1.42g (91%). ^{1}H NMR ($CDCl_3$) δ 6.432 (s, 1H), 3.971 (t, 2H), 3.901 (s, 3H), 3.791 (s, 3H), 3.778 (s, 3H), 3.673 (s, 3H), 2.414 (t, 2H), 2.218 (s, 3H), 1.808 (m, 4H).

Synthesis of 28: Pyridine-2, 6-dicarboxylate (0.25g, 1.5mmol) was added to a cold (0°C) solution of **27** (0.31g, 1mmol) in 20mL of 7:3 acetonitrile/water. An ice cold solution of ceric ammonium nitrate (CAN) (0.82g, 1.5mmol) in 20mL of 1:1 acetonitrile/water was slowly added over 20 minutes. The mixture was stirred at 0°C for 20 minutes and poured into 40mL of water and extracted with methylene chloride. The organic layers was collected, dried over $MgSO_4$, followed by quick solvent removal under reduced pressure and quickly transferred to silica gel flash column chromatography using 4:1 hexanes/ethyl acetate as eluent. Yield 0.14g (57%). ^{1}H NMR ($CDCl_3$) δ 6.431 (s, 1H), 4.195 (t, 2H), 3.988 (s, 3H), 3.679 (s, 3H), 2.399 (t, 2H), 2.035 (s, 3H), 1.785 (m, 4H).

Synthesis of 29: A mixture of **28** (0.14g, 0.5mmol) and 1g of undecanoyl peroxide in 20mL of glacial acetic acid was heated at 95°C for 14 hours. The mixture was added heptane to remove the acetic acid *in vacuo*. The resultant slurry was re-dissolved in

diethyl ether and washed with 0.5M $NaHCO_3$ and water. The ether solution was dried over magnesium sulfate. After removal of the solvent, the residue was separated with silica gel flash column using 1% acetic acid in 4:1 hexanes/ethyl acetate. Yield 67mg (34%). ^1H NMR ($CDCl_3$) δ 4.14 (t, 2H), 3.974 (s, 3H), 3.665 (s, 3H), 2.50 (t, 2H), 2.34 (m, 2H), 1.998 (s, 3H), 1.88 (m, 2H), 1.65 (m, 2H), 1.68 (m, 2H), 1.28 (m, 14H), 0.90 (t, 3H).

*Synthesis of **A2-2***: To a solution of **29** (0.5mmol) in 10mL pyridine was added LiI (0.14g, 1mmol). The mixture was heated at 90°C and monitored by TLC. After the starting material disappeared, the reaction was quenched with water, washed with 0.01N HCl solution and extracted with diethyl ether. The ether solution was dried and concentrated *in vacuo*. The residue was separated with silica gel flash column chromatography using 1% acetic acid in 4:1 hexanes/ethyl acetate. Yield 190mg (92%). ^1H NMR ($CDCl_3$) δ 4.11 (t, 2H), 3.974 (s, 3H), 2.50 (t, 2H), 2.35 (m, 2H), 1.998 (s, 3H), 1.82 (m, 2H), 1.64 (m, 2H), 1.68 (m, 2H), 1.28 (m, 14H), 0.90 (t, 3H).

*Synthesis of **30a,b***: A solution of decylubiquinone (1g, 3.1mmol) in anhydrous 20mL THF was added 2mL ethanethiol. The mixture was cooled to –42°C. Anhydrous $AlCl_3$ powder (2.13g, 16mmol) was added into the solution under argon flow. The mixture was stirred for 5 minutes and quenched with 15mL water. $CuCl_2$ aqueous solution was added to precipitate ethanethiol. The solid was filtered off and the liquid phase was extracted with diethyl ether. The ether solution was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The product was purified by silica gel flash column

chromatography using 5:1 hexanes/ethyl acetate. Yield 0.80g (84%). ^{1}H NMR ($CDCl_3$) δ 6.49 (bs, 1H), 4.064 (s, 3H), 2.464 (t, 2H), 2.04 (s, 3H), 1.56-1.20 (m, 16H), 0.88 (t, 3H)

*Synthesis of **31a,b***: To a mixture of **30a,b** (0.62g, 2mmol) and TBDMS chloride (0.34g, 2.2mmol) in anhydrous methylene chloride (20mL), triethyl amine (0.23g, 2.3mmol) was added. The solution turned to dark red color right away. The reaction was allowed to continue for overnight under stirring and then poured into dilute hydrochloric solution. The mixture was extracted with diethyl ether and the organic layers were collected, combined, dried over magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography using 9:1 hexanes/ether as eluent. Yield 0.78g (92%). ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H), 2.44 (t, 2H), 2.00 (s, 3H), 1.4-1.2 (m, 16H), 0.985 (s, 9H), 0.874 (t, 3H), 0.225 (s, 6H).

*Synthesis of **32a,b***: TBAF (1.1mL of 1M solution in THF) was added 20mL anhydrous acetonitrile twice, dried with rotary evaporator twice and re-dissolved in 3mL anhydrous acetonitrile. To a mixture of **31a,b** (0.42g, 1mmol) and *tert*-butyl bromoacetate (0.79g, 4mmol) in 20mL anhydrous acetonitrile, KF-alumina (0.3g) and TBAF acetonitrile solution was added under argon flow. The mixture was stirred for overnight under argon atmosphere. The solid was filtered off and the liquid was concentrated. The residue was separated by silica gel flash column chromatography using 4:1 hexanes/ethylacetate as eluent. Yield 0.39g (92%). ^{1}H NMR ($CDCl_3$) δ 4.842 & 4.712 (s, 2H), 4.003 & 3.788 (s, 3H), 2.43 (m, 2H), 2.102 (s, 3H), 1.6-1.2 (m, 16H), 1.41 (s, 9H), 0.880 (t, 3H).

Synthesis of A23: Compound **32a,b** (0.22g, 0.5mmol) was dissolved in 10mL 1:1 TFA/CH_2Cl_2 ice-cold solution. The mixture was stirred at 0°C and TLC was used to monitor the progress of the reaction until all the starting material was converted to product. The solvent was removed *in vacuo* and the residue was separated with silica gel flash column chromatography using 1% acetic acid in 4:1 hexanes/ethyl acetate. Yield 0.18g (97%). ^{1}H NMR ($CDCl_3$) δ 4.837 & 4.753 (s, 2H), 3.997 & 3.782 (s, 3H), 2.43 (t, 2H), 1.995 (s, 3H), 1.7-1.1 (m, 16H), 0.870 (t, 3H).

Synthesis of 33: To a mixture of *tert*-butyl BCMB (47.2mg, 0.1mmol) and **A5-2** (38mg, 0.1mmol) in anhydrous dichloromethane (10mL), 1,3-dicyclohexylcarbodiimide (DCC) (0.12mL of 1.0M DCC solution in dichloromethane) was added under argon flow, followed by the addition of DMAP (10mg). The mixture was stirred under argon for 20 hours, washed with sodium bicarbonate solution, water, dilute HCl solution and water again. The organic phase was dried over magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography using 4:1 hexanes/ethyl acetate as eluent. Yield 76mg (89%). ^{1}H NMR ($CDCl_3$) δ 7.90 (m, 2H), 7.534 (m, 1H), 7.42 (m. 2H), 6.51 (d, 2H), 6.37 (t, 1H), 5.81 (s, 1H), 4.42 (s, 4H), 3.992 (s, 6H), 2.77 (t, 2H), 2.52 (t, 2H), 2.47 (t, 2H), 1.8-1.1 (m, 16H), 1.458 (s, 18H), 0.878 (t, 3H).

Synthesis of 34: Compound **33** (43mg, 0.05mmol) was dissolved in ice-cold 1:1 TFA/CH_2Cl_2 (10mL) placed in an ice bath. The mixture was stirred at 0°C and TLC was used to monitor the progress of the reaction until all the starting material was converted to the product. The product was purified by silica gel flash column chromatogrphy using

1% acetic acid in 4:1 hexanes/ethyl acetate as eluent. Yield 36mg (96%). ^{1}H NMR ($CDCl_3$) δ 7.90 (m, 2H), 7.534 (m, 1H), 7.42 (m. 2H), 6.51 (d, 2H), 6.37 (t, 1H), 5.81 (s, 1H), 4.38 (s, 4H), 3.992 (s, 6H), 2.77 (t, 2H), 2.52 (t, 2H), 2.47 (t, 2H), 1.8-1.1 (m, 16H), 0.878 (t, 3H).

Synthesis of Thallium Salt of N-hydroxy-2-thiopyridone: N-hydroxy-2-thiopyridone (1.27g, 10mmol) was dissolved in 100mL anhydrous diethyl ether. Thallium ethoxide (2.5g, 10mmol) was added under rigorous stirring. After an hour, the suspension was filtered. The solid was collected and dried *in vacuo*.

Synthesis of N-hydroxy-2-thiopyridone caged decylubiquinol (HT-DQ): A 50 mL round bottom flask was charged with decylubiquinone (0.1g, 0.3 mmol) dissolved in 5 mL diethyl ether. Sodium dithionite (1 mmol) was dissolved in 10mL water and was then quickly added into the ubiquinone-2 solution. The solution was stirred rigorously under argon flow until the red color of ubiquinol disappeared. Hexanes (20 mL x 3) were used to extract the slurry and the organic phase was collected, dried with magnesium sulfate and concentrated to give a colorless oil. The colorless oil was further dried with anhydrous diethyl ether and re-dissolved in 30 mL anhydrous THF. The solution was charged with argon flow and cooled to –70°C. 2.5M n-butyllithium hexane solution (120 μL) was added dropwise. The solution was stirred at this temperature for 10 minutes and then slowly warmed up to 0°C and stirred for another 20 minutes at 0°C. Triphosgene (0.1mmol) was added first and then 0.3mmol pyridine was added dropwise. The solution was allowed to react for another 6 hours. Thallium salt of N-hydroxy-2-thiopyridone

(1.2g, 0.4mmol) was added and the suspension was rigorously stirred for 2 hours and then the solid was filtered off. The solid was rinsed with diethyl ether a few times and the organic solution was collected and combined with filtrate solution. The organic solution was dried *in vacuo*. TLC indicated a single spot. Yield 98mg (71%). ^{1}H NMR ($CDCl_3$) δ 8.08 (d, 1H), 7.69 (d, 1H), 7.28 (m, 1H), 6.78 (m, 1H), 3.78 (s, 6H), 2.56 (m, 2H), 2.134 (s, 3H), 1.43 (m, 2H), 1.28 (m, 14H), 0.86 (t, 3H).

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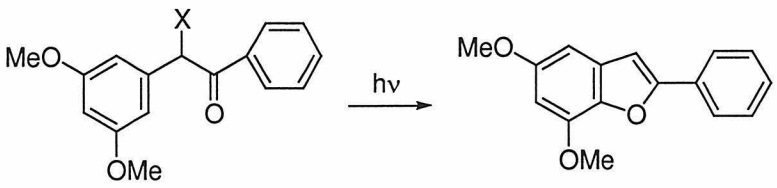
Chapter 6

Study on the Photolysis Mechanism of Benzoin Esters

Introduction

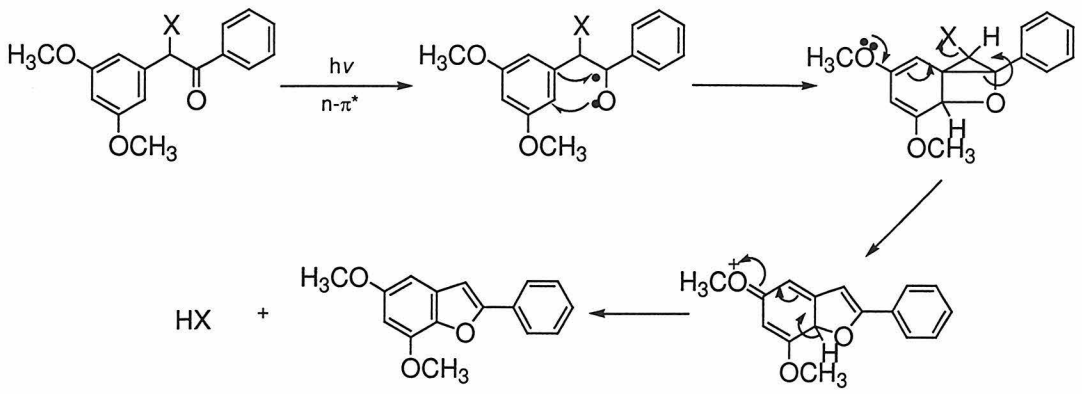
As a photolabile protecting group, 3', 5'-dimethoxybenzoin (3', 5'-DMB) possesses remarkable photolysis properties including fast photolysis rate, high quantum yield (up to 0.78) and non-reactive photolysis by-product[10,9 8]. It can release a variety of functional groups with 300-360 nm irradiation [1,13,5,6] (Scheme 6.1). Therefore, it has found applications in a wide variety of disciplines. However, the mechanism of DMB photolysis still remains in debate.



X = $NHR_2^+Cl^-$, OCOR, Cl, OTs, OCO_2R, OCONHR, $OPO(OR)_2$, OPO_3^{2-}, etc.

Scheme 6.1. Photolysis of 3', 5'-Dimethoxybenzoin Compounds

Sheehan et al.[11]proposed that a Paterno-Buchi reaction between the singlet n-π* photoexcited carbonyl group of the acetophenone and the 3', 5'-dimethoxybenzene results in the formation of a strained bicyclic intermediate. Subsequent ring opening with the loss of the acetate ion leads to the benzofuran photoproduct. The electron-donating substituents, methoxy groups, at *meta* positions facilitate the electrophilic attack of the n-π* carbonyl oxygen (Scheme 6.2).

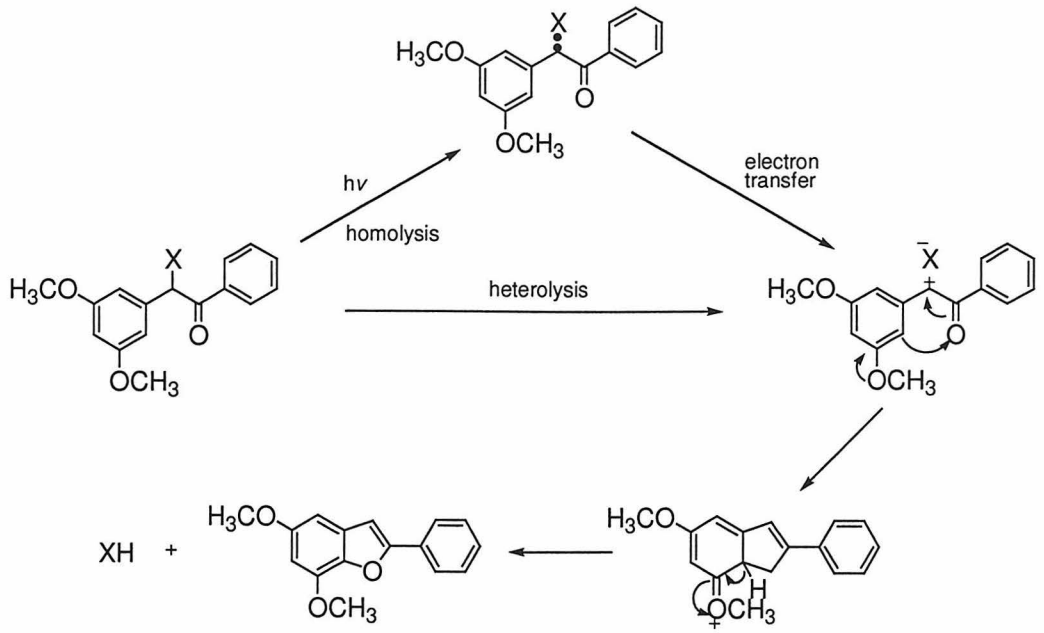


Scheme 6.2. Photolysis Mechanism Proposed by Sheehan et al.

Another proposed mechanism[2] suggested that photoexcitation leads to initial homolysis of the C-X bond, followed by electron transfer to generate an α-keto cationic intermediate. The formation of the cationic intermediate resulted from photoexcitation is supported by Zimmerman's study[14,15] on the electronic influence of *meta* donor substituents on the benzene excited states. In their study, Zimmerman et al. concluded that solvolysis of benzylic derivatives is promoted by *m*-methoxy groups. In addition, the intramolecular electrophilic addition to aromatic ring was also facilitated by the *meta*-methoxy groups that are located *ortho/para* to the site of electrophilic attack of the carbonyl (Scheme 6.3). In addition to the above postulation, a direct C-X bond heterolysis to form the α-keto cationic intermediate has also been proposed[7].

However, the absorption spectra of the dimethoxybenzoins raised questions concerning the basis of the excitation of these groups. Their major absorption is not at the 350nm wavelength used for photoactivation. As a matter of fact, the substituted aromatic

ring that was postulated to promote photosolvolysis does not absorb this far to the red. If



Scheme 6.3. Photosolvolysis of DMB Compounds

the photosolvolysis mechanism is valid, there must be a special influence of the benzoyl group on the reactivity of the benzylic ester. One possibility is the initial absorption of benzoy group followed by energy transfer.

With the growing interest of 3', 5'-DMB in applied photochemistry, it becomes more important to understand its photolysis mechanism in detail. The mechanistic knowledge would direct our design of photo-reactive compounds based on benzoin structure for novel or improved photochemical properties. In my research attempts to probe the photolysis mechanism of 3', 5'-DMB, two approaches were employed: one is to examine the substitution impact on the photolysis properties of benzoin esters; the other is to simulate the photolysis of benzoin acetates by *ab initio* quantum chemistry

calculation.

Result and Discussion

Substitution Influence on the Photolysis of Benzoins

In his pioneering study, Sheehan[10,9] discovered that the photolytic cyclization of benzoin compounds is influenced by many factors, such as the nature of the leaving group, the substitution of the phenyl (or other aromatic) groups and the solvent used in the photolysis. In most applications, the leaving group and the solvent are pre-determined as required by the specific application. It is the substitution on the phenyl rings that can be modified to achieve the photochemical or other properties desired. In addition, the substitution influence on the photolysis property of a cage compound contains valuable information on the structure-property relationship[5]. A systematic study in this aspect could not only reveal mechanistic knowledge, but also provide a guideline for predicting photolysis property of novel benzoin structures.

We studied a series of 3′, 5′-dimethoxybenzoins whose benzoyl groups were modified to more delocalized conjugated systems or were attached with strong electron-donating group (Figure 6.1). The synthesis of these compounds was based on the *Corey-Seebach* dithiane addition[12]. Scheme 6.4 illustrates the general procedure for the synthesis of these compounds.

In the photolysis study with Excimer laser (308nm), all of the compounds **1 - 5** showed a smooth photolysis and a clean isobestic point before photobleached. The photoproducts were separated and the formation of photocyclization products was

confirmed. The percentage of photolysis vs. the number of laser pulse shots for each compound was obtained, as shown in Figure 6.2. This study indicated that the photolysis of compounds **2** – **5** was not as efficient as that of 3′, 5′-dimethoxybenzoin acetate **1**. On the other hand, although the UV-Vis absorption spectra of compounds **2** – **5**

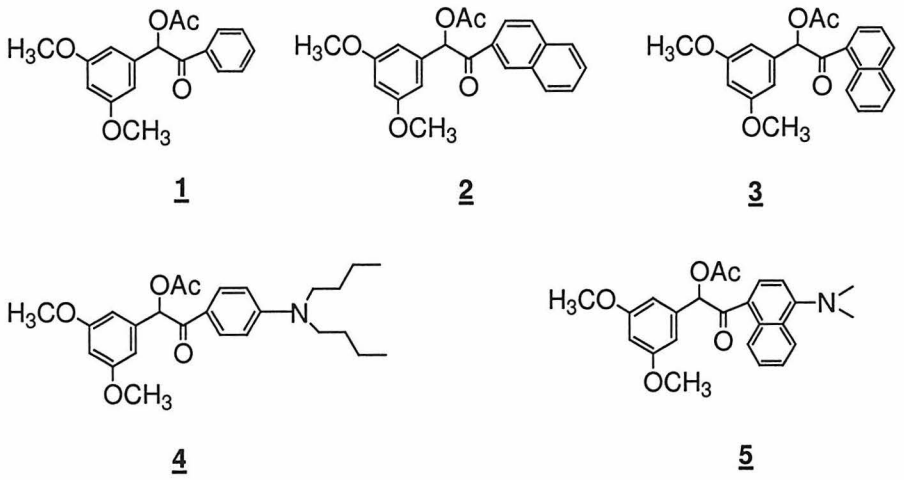
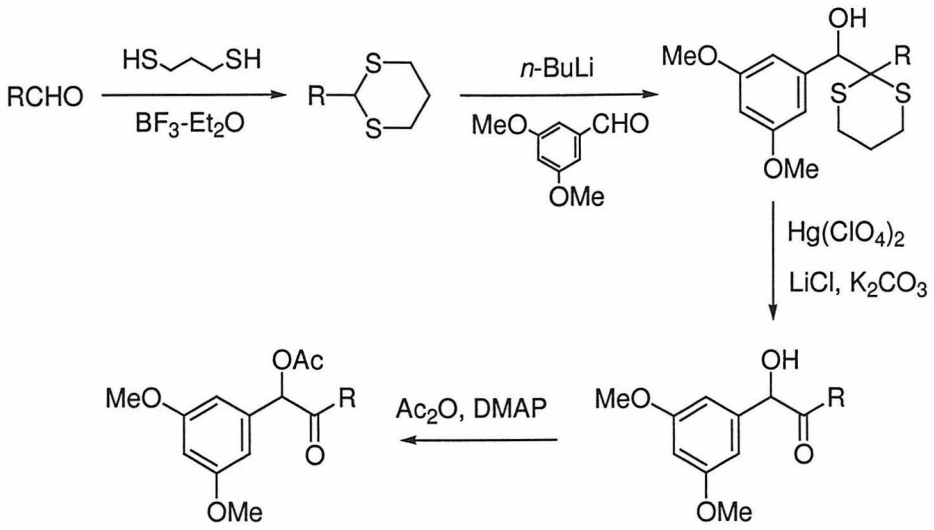


Figure 6.1. Dimethoxybenzoin Derivatives (I)



Scheme 6.4. Synthesis of Benzoin Acetates 1 – 5

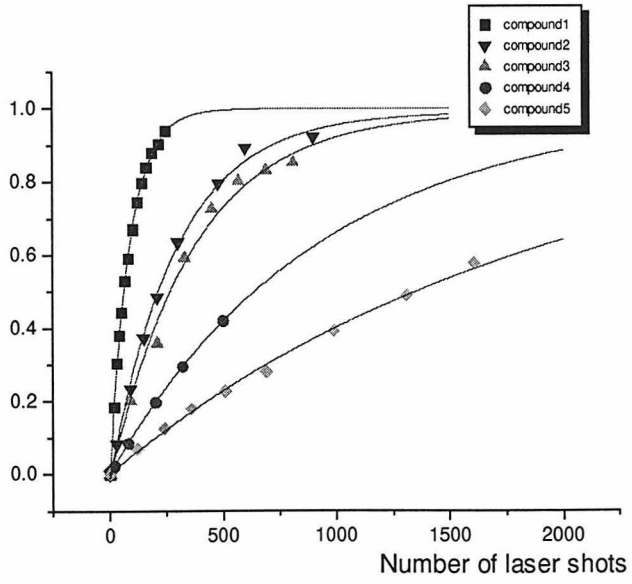


Figure 6.2. Comparison of the Photolysis of Compound 2 – 5

showed strong absorption at 300-360 nm than that of 3′, 5′-dimethoxybenzoin acetate **1**, compounds **2** – **5** do not produce photolytic cyclization product in high yield when illuminated with light of >350nm wavelength.

These observations suggested that carbonyl group plays an important role in the photolytic cyclization reaction. Since the carbonyl and the phenyl ring are conjugated in benzoyl, either changing the phenyl ring or attaching an electron-donating group will change the electron density on carbonyl group, and consequently change its photo-reactivity. One possibility is that the amino group or the larger aromatic ring delocalizes the electronic disturbance on the carbonyl carbon of the n → π* state, resulting in the less photosensitive benzoins.

In another series of benzoin derivatives, benzoyl groups were attached with electron-withdrawing functional groups (Figure 6.3). The *Corey-Seebach* dithiane

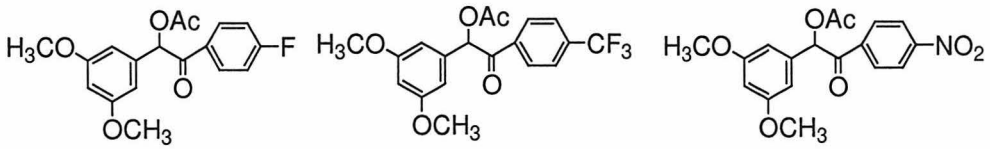
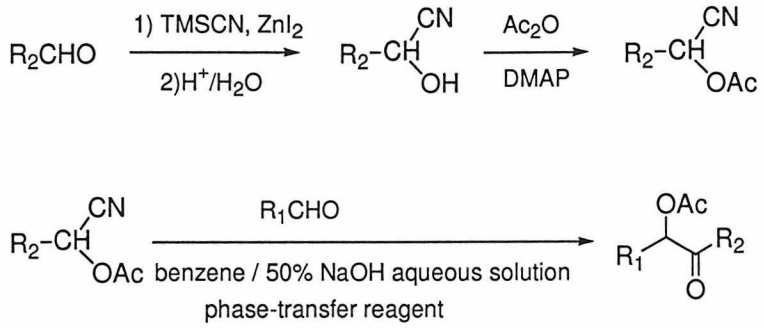


Figure 6.3. Dimethoxybenzoin Derivatives (II)

addition reaction can not be employed for the synthesis of these compounds due to the

side reaction of *n*-butyllithium reagent with those electron-withdrawing groups. Therefore, a new synthetic method was developed (See chapter 7 for detailed description) as shown in Scheme 6.5. Photolysis of these compounds was performed with arc lamp and the photolysis absorption spectra were shown in Figures 6.4 – 6.7. The photolysis result showed that the photolysis efficiency and photo-cyclization yield of compound **6** and **7** are comparable with those of 3', 5'-DMB. Only compound **8** showed lower photolysis efficiency and lower yield of photo-cyclization.



Scheme 6.5. Synthesis of Benzoin Acetates 6 – 8

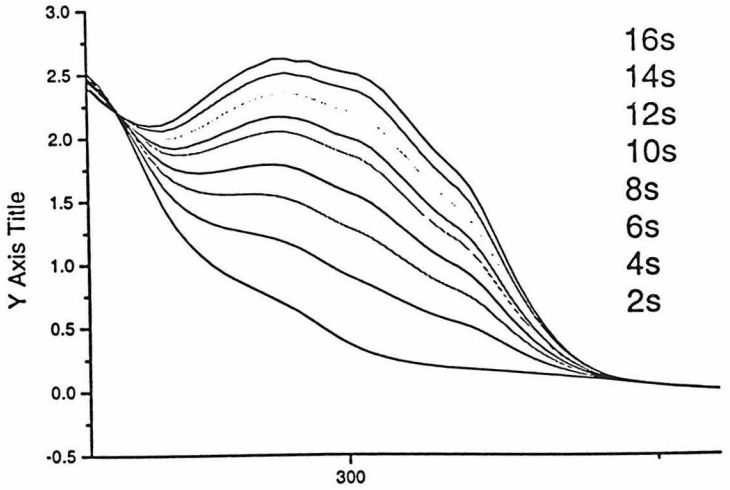


Figure 6.4. Absorption Spectra of the Photolysis of Compound 6

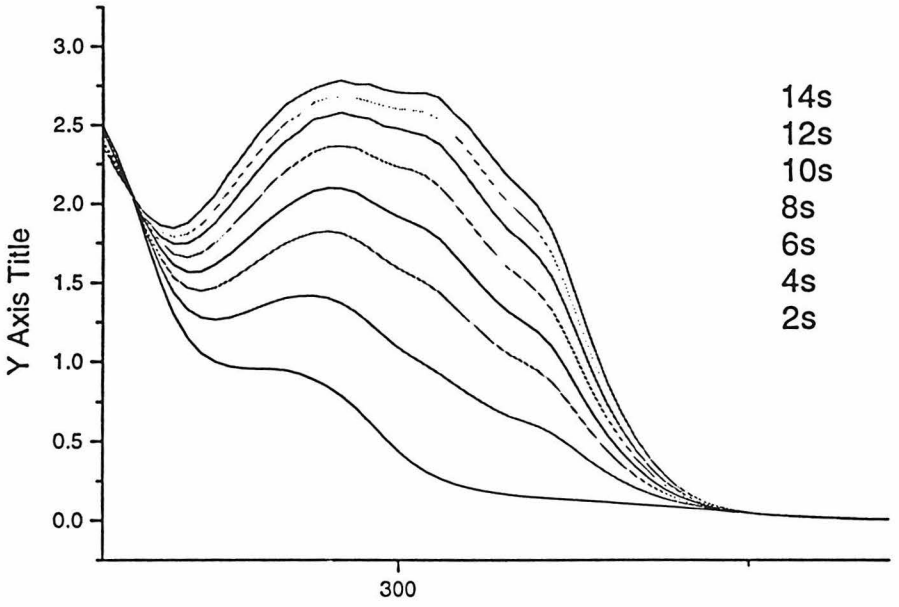


Figure 6.5. Absorption Spectra of the Photolysis of Compound 1

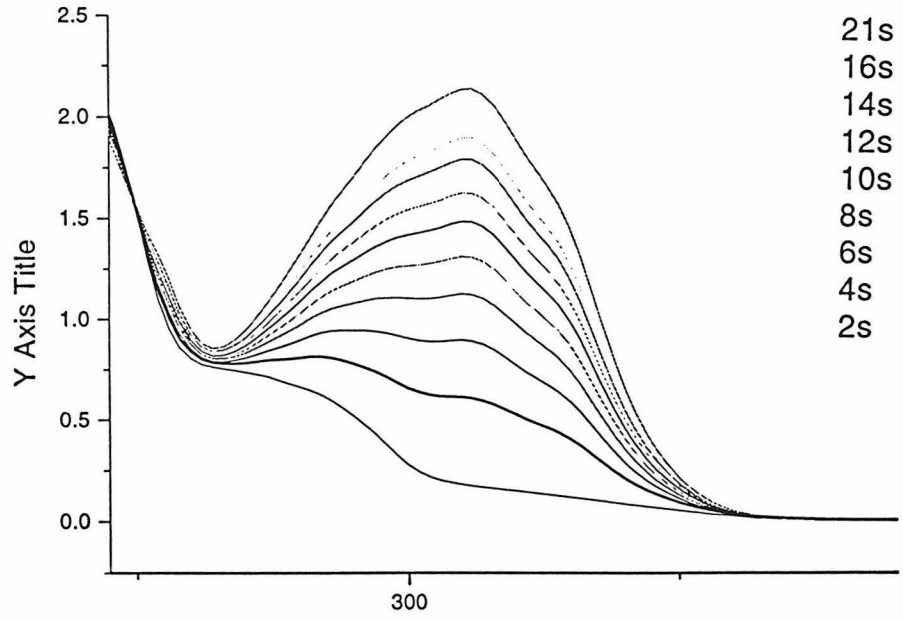


Figure 6.6. Absorption Spectra of the Photolysis of Compound 7

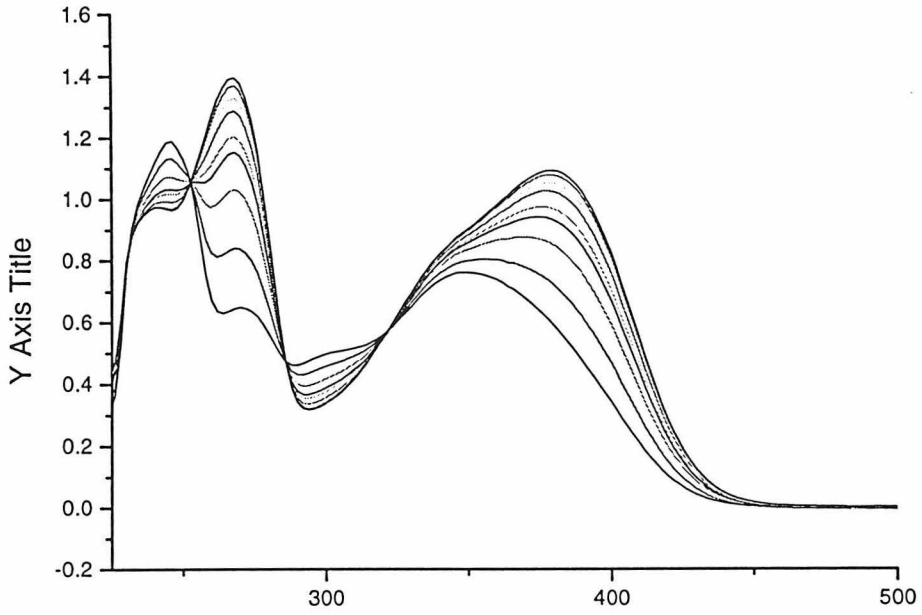
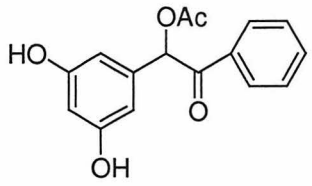


Figure 6.7. Absorption Spectra of the Photolysis of Compound 8

Solvent Influence on the Photolysis of Benzoins

Solvent effect was also investigated in the photolysis of 3', 5'-DMB and compound **9** (Figure 6.8). The procedure used for the synthesis of compound **1 - 5** was followed for the synthesis of **9**. Figure 6.9 shows the solvent effect on the photolysis of 3', 5'-DMB and Figure 6.10 shows the photolysis of compound **9** in THF and methanol. The fact that polar solvent accelerates the formation of benzofuran product suggested that the photolysis occur via an ionic intermediate (or a heterolysis step) because solvation tends to lower the energies of the ion pairs relative to the radical pairs in the polar solvents. The dramatic difference of the compound **9** photolysis in THF and methanol stems from the interaction between methanol and the two hydroxy groups on the benzene ring. This interaction may result in a change in the electron-donating capability of the

hydroxy group and consequently change the electron configuration of the benzene ring.



9

Figure 6.8. 3', 5'-Dihydroxybenzoin Acetate

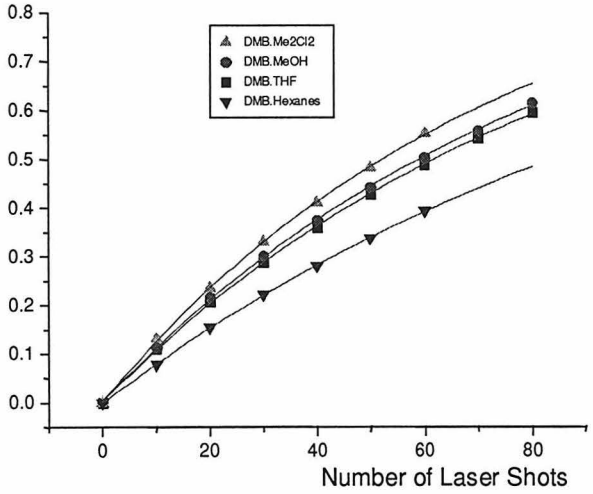


Figure 6.9. Solvent Effect of 3', 5'-DMB Photolysis
(Y axis is the percentage of photolytic cyclization product.)

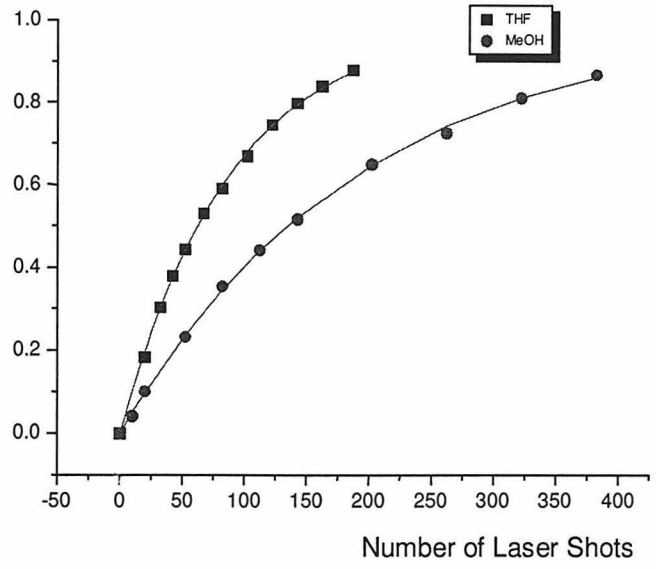


Figure 6.10. Photolysis of Compound 9 in THF and Methanol
(Y-axis is the percentage of photolytic cyclization product.)

Quantum Chemistry Calculation of the Photolysis of Benzoins

In order to obtain a better understanding of the photolysis mechanism of benzoin esters, a quantum chemistry calculation was performed to simulate the photolysis of 3′, 5′-DMB acetate. Hartree-Fock wave functions and the 6-31G** basis set were used in this calculation. An open-shell singlet wave function was used for the excited state, in which a single-excitation from orbital a into orbital b is described by the wave function:

$$\Psi_a^b = \Psi_{core}[\ \varphi_a\varphi_b + \varphi_b\varphi_a\ ](\alpha\beta - \beta\alpha)$$

where ψ_{core} represents a core wave function consisting of doubly-occupied orbital, and α and β represent the spin-up and spin-down spin wave functions. These calculations were performed using the Jaguar Program Suite, version 3.5.

Gas Phase Simulation

The ground state structure of 3', 5'-dimethoxybenzoin (3', 5'-DMB) acetate was optimized by applying the Hartree-Fock method and 6-31G** basis set, with a total SCF energy of –1066.43 Hartrees and a dipole moment of 3.97 Debye.

It was reported that triplet quenchers, such as methylnaphthalene or piperylene, could not appreciably quench the photolysis of 3', 5'-DMB acetate at concentrations as high as 0.15M. This suggests that the photolysis of 3'5'-DMB acetate is a singlet reaction. Therefore, Open Shell Singlet (OSS) excitation was applied to the ground state 3'5'-DMB acetate. From the ground state, it is excited to the OSS excited state without geometry optimization. The energy of the excited state is –1066.24 Hartrees and the dipole moment is 17.83 Debye. From molecular orbital analysis, this process could be regarded as a π-π^* excitation, in which one electron was transferred from the HOMO (π orbital) located on substituted benzene ring to the LUMO (π^* orbital) located on carbonyl double bond. This π-π^* excitation disrupted the carbonyl double bond and the aromaticity of the benzene ring as suggested in Natural Bond Analysis. This electron transfer observation was also supported by atomic charge analysis in which significant atomic charge changes were found at C4 that loses about half electron and the carbonyl double bond that gains about half electron.

Following the excitation, the geometry of the excited 3', 5'-DMB acetate was allowed to relax. It was found that upon the geometrical relaxation, the carbonyl oxygen moves toward the *ortho* position of carbinol group and forms a single bond, closing the five-membered ring. This spontaneous ring closure could be regarded as a nucleophilic attack of the carbonyl oxygen on the aromatic ring. Upon the photolytic cyclization, the system energy decreases by 49.21 kcal/mol. Based on both molecular orbital and NBO analysis, this cyclic intermediate is a biradical species (Figure 6.11).

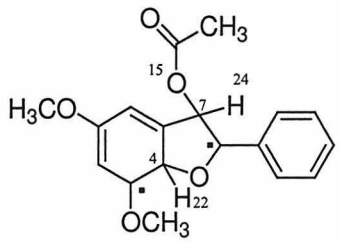
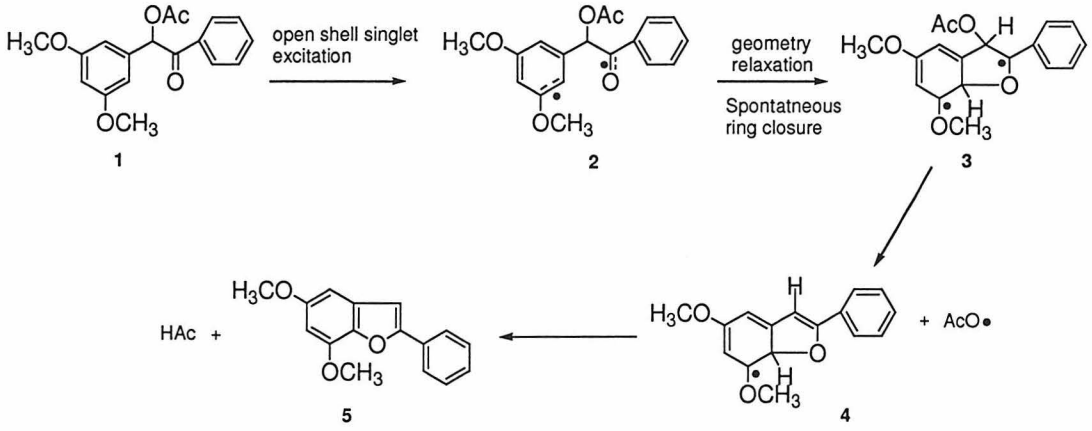


Figure 6.11. Photolytic Cyclization Intermediate

The biradical intermediate will continue to reorganize to restore aromaticity. The neutralization might occur by losing H22 from atom C4 and either H24 or CH_3O from atom C7. Each pathway was investigated by calculating the bond cleavage energies (Table 6.1). It was found that cleaving C7-O15 bond is energetically favored, which indicated that the acetate will leave first, followed by the bond cleavage C4-H22 that allows the system to regain aromaticity. The photolysis pathway and energetic profile are summarized below (Scheme 6.6).

Bond	Bond Cleavage Energy in Gas Phase (kcal/mol)
C7-O15	45.77
C7-H24	81.06
C4-H22	63.29

Table 6.1. Bond Cleavage Energies



Scheme 6.6. Photolysis Pathway in Gas Phase

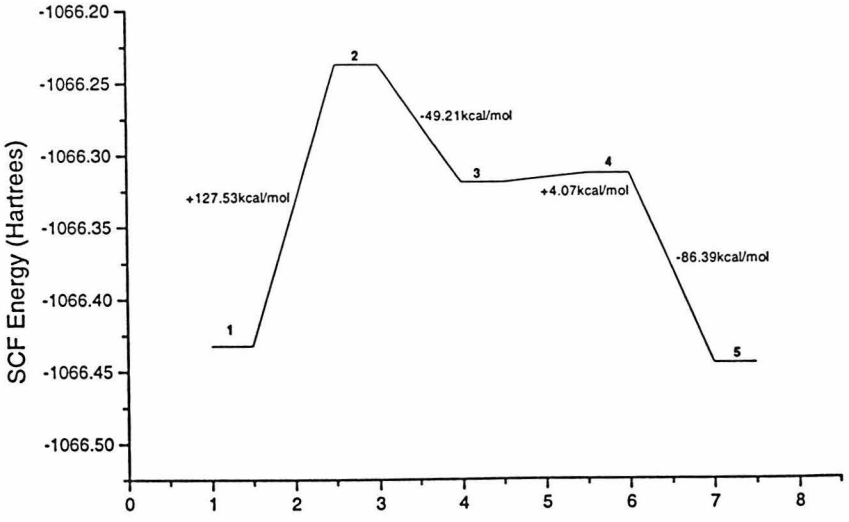


Figure 6.12. Energetically Profile for Gas Phase Simulation of Photolysis

Solvation Effect on the Photolytic Cyclization of 3', 5'-DMB Acetate

Solvation effect was further considered in the photolysis simulation. It is found that a cationic intermediate (leaving group is the acetate ion) gave a more energetically favorable profile. In order to find the bonding state of this cationic intermediate, Natural Bond Orbital (NBO) analysis based on the intermediate's electronic configuration was carried out. The structure from the NBO analysis is shown in Figure 7.5. In contrast to the energy profile obtained when the leaving group is acetate radical in which system energy increase 4.07kcal/mol in the corresponding step, the system energy goes down 31.74 kcal/mol in CH_2Cl_2 with a cationic intermediate.

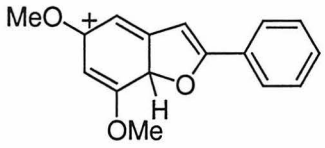
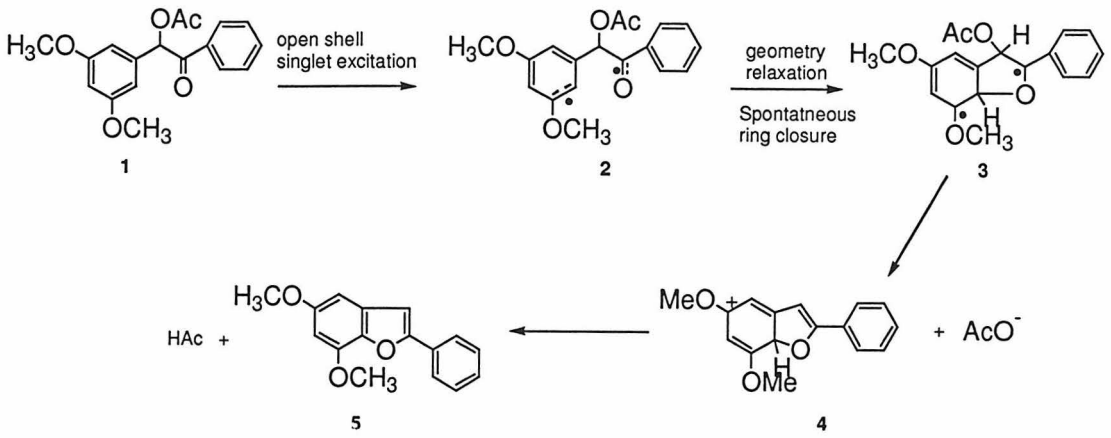


Figure 6.13. Cationic Intermediate

In the final step, the loss of a proton H22 led the molecule to regain its aromaticity and lowered the system energy by 48.36 kcal/mol. The results of simulation are summarized below (Scheme 6.7 and Figure 6.14).



Scheme 6.7. Pathway of Photolysis in Dichloromethane

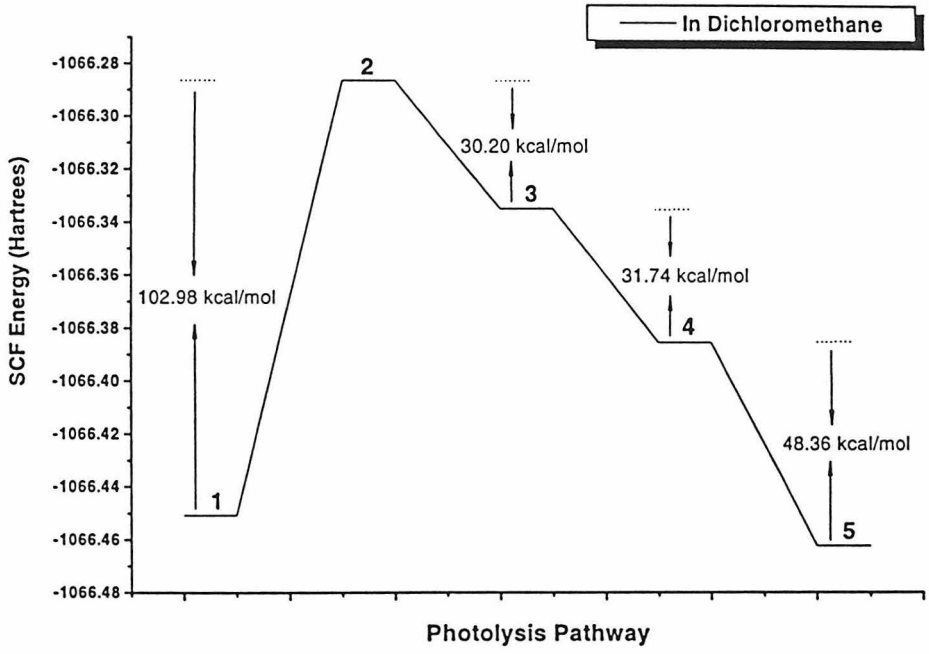
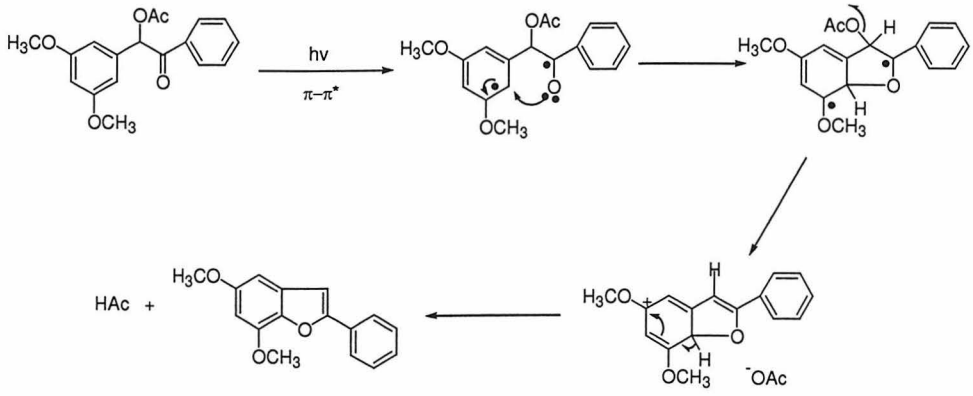


Figure 6.14. Photolysis Pathway and Energetic Profile for Solvation Simulation

Based on the calculation, a new photolysis mechanism of 3', 5'-dimothxybenzoin acetate was proposed, as shown in Scheme 6.8. It is based on molecular orbital analysis. The $\pi \rightarrow \pi^*$ excitation is not the conventional $\pi \rightarrow \pi^*$ excitation in which both π and π^* are located on the same benzene ring. The electron transfer between the benzoyl and the benzene ring is critical for the cyclization.



Scheme 6.8. Proposed Mechanism of 3', 5'-DMB Ester Photolysis

Simulation on the Photolysis of Other Benzoin Derivatives

To probe the structure-property relationship that governs the photochemical properties of benzoins, the simulation method developed above also applied to the photolysis of a series of benzoin compounds (Figure 6.15).

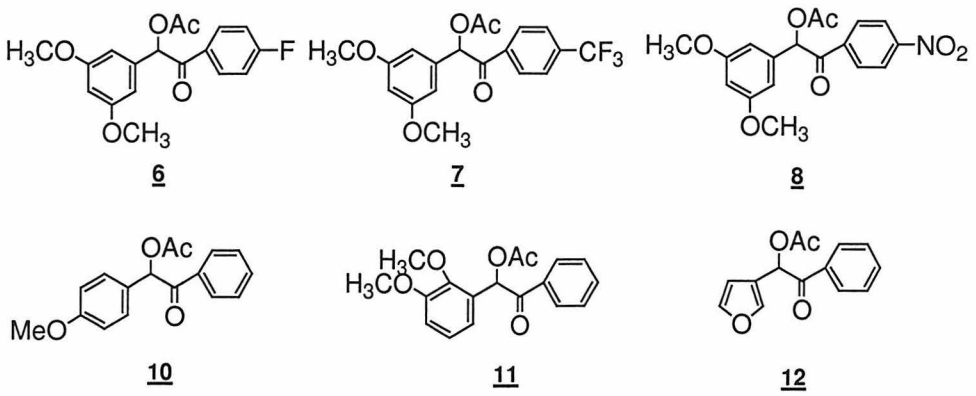


Figure 6.15. Benzoin Acetates Studied in Simulation

From our calculation, the spontaneous photolytic cyclization upon geometrical relaxation from the open shell singlet excited state was observed for compound **6**, **7**, **11, 12**. In addition, the molecular orbital, NBO and atomic charge analysis indicated the similar photolysis pathway as compared with 3', 5'-dimethoxybenzoin acetate. It has been reported before that the photolysis of compound **11**, 2', 3'-dimethoxybenzoin (2', 3'-DMB) acetate leads to corresponding benzofuran product in high yield. Our calculation result is consistent with this reported experimental observation. The simulation results are also consistent with our experimental observations on the photolysis of compounds **6,7.** Attempt has been made to synthesize compound **12** using the dithiane addition reaction but failed to yield the desired product.

In our calculations on the photolysis of compound **8** and **10**, no photolytic cyclization was observed. In experiment, photolysis of compound has been reported to give a low yield of benzofuran (~10%) and a mixture of radical recombination products.

Our calculation showed that although the LUMO of *p*-methoxybenzoin is located on the carbonyl, its HOMO is located on the benzoyl aromatic ring, not on the substituted benzene ring as that in 3', 5'-DMB or 2', 3'-DMB acetate. Therefore, the excitation of electron from HOMO to LUMO of *p*-methoxybenzoin acetate does not lead to the electron transfer from the substituted benzene ring to the benzoyl, which is critically important for the photolytic cyclization based on our simulation. On the other hand, the second HOMO of *p*-methxybenzoin is located at the substituted benzene ring as the HOMO of 3', 5'-DMB or 2', 3'-DMB acetate. The electron transfer from the second HOMO to LUMO may form benzofuran if the input energy is high enough for this excitation.

For compound **8,** NBO and molecular orbital analysis indicated that although the electron transfer is facilitated by the electron-withdrawing nitro group in the benzoyl ring, the transferred charge is delocalized in the benzoyl ring. Therefore, the nucleophilic attack of the carbonyl oxygen on the substituted benzene ring is disfavored energetically. In photolysis experiment, it has been observed that compound **8** yielded the cyclic photoproduct less efficiently than 3', 5'-DMB acetate.

From the above comparisons, it seems that the simulation is strikingly consistent with experimental results. It has to be pointed out that there exist significant mechanistic differences for benzoins with different structure (substitution). One striking example is the 3', 5'-DMB and the unsubstituted benzoin: the photolysis of DMB carboxylates and phosphates is unaffected by triplet quenchers[9,5], whereas photolysis of unsubstituted benzoin carboxylates and phosphates is quenched[10,3,4]. In the calculations described

above, only singlet photoreaction was considered. The simulation did not establish a guideline yet to predict which photolysis pathway will be taken for a specific benzoin structure. Even for singlet photoreaction, only the excitation from HOMO to LUMO was considered. It did not exclude the possibility that excitation across other energy levels may occur.

Therefore, caution must be taken in using the computer simulation to predict the photolysis properties of a new benzoin compound. In general, those compounds predicted by the simulation to be able to photolytically cyclize tend to be photolyzed rapidly and tend to render high yield of cyclic photoproduct in experiment. Those compounds that were predicted to fail to photolytically cyclize by the simulation tend to give a low yield of cyclization product and yield a slower and less efficient photolysis. However, it does not rule out the possibility of the formation of cyclization product via other photolysis pathways, though it may be less efficient.

Materials and Methods

General: 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. ^{1}H NMR spectra were recorded on a GE QE300 spectrometer operating at a nominal frequency of 300 MHz. The optical absorption was monitored with a HP 8452 diode array spectrophotometer. Steady-state photolysis was performed with an Oriel 66011 Hg vapor arc lamp operating at 450 W and filtered through water-cooled Schott glass type UG11 and WG320 filters. Laser

spectroscopy was performed with a Lambda Physik LPX201I XeCl excimer laser (308 nm), with its energy in the range of 1.7-2.0 mJ/pulse.

Preparation of aldehyde dithiane (Scheme 6.4). A flame dried round-bottom flask was charged with a stir bar, aldehyde (5mmol), propane dithiol (5.5mmol) and dichloromethane, cooled to –20°C. Borontrifloride diethyl ether (12mmol) was added slowly via syringe. The mixture was allowed to warm up to room temperature under vigorous stirring. TLC was used to monitor progress of the reaction until the complete consumption of starting material. The solution was poured into water. The organic phase was washed with water, sodium hydroxide solution and water again, dried over magnesium sulfate and concentrated under reduced pressure. The residue was separated by silica gel flash chromatography. Proton NMR data for the dithiane intermediate of compound **2** – **5** were listed below.

For compound **2**: 7.96 (s, 1H), 7.84 (d, 3H), 7.60 (dd, 1H), 7.47 (m, 2H), 5.37 (s, 1H) 3.01 (m, 4H), 2.06 (m, 2H).

For compound **3**: 8.45 (d, 1H), 7.99 (d, 1H), 7.80 (d, 1H), 7.51 (d, 1H), 7.48-7.36 (m, 3H), 5.82 (s, 1H), 3.03 (m, 4H), 2.07 (m, 2H).

For compound **4**: 3.05 (t, 4H), 1.29 (m, 8H), 0.85 (t, 6H), 6.75 (d, 2H), 5.45 (s, 1H), 7.20 (d, 2H), 3.15-2.85 (m, 4H), 2.25-1.85 (m, 2H).

For compound **5**: 8.27 (dd, 2H), 7.716, 7.690 (d, 1H), 7.56-7.47 (m, 2H), 7.031-7.057 (d, 1H), 5.865 (s, 1H), 2.93-3.26 (m, 4H), 2.882 (s, 6H), 1.90-2.30 (m, 2H).

Preparation of compounds **1** – **5** from the corresponding aldehyde dithiane followed the procedure as described in chapters 3 and 4. For preparations of compounds **6** – **8**, please refer to the experimental section of chapter 8. Proton NMR data were listed below.

Compound **1**: 7.94 (d, 2H), 7.45 (m, 3H), 6.85 (s, 1H), 6.60 (d, 2H), 6.405 (t, 1H), 3.739 (s, 6H), 2.10 (s, 3H).

Compound **2**: 8.506 (s, 1H), 7.999-7.809 (m, 4H), 7.56-7.50 (m, 2H), 6.937 (s, 1H), 6.663 (d, 2H), 6.405 (t, 1H), 3.792 (s, 6H), 2.241 (s, 3H).

Compound **3**: 8.69 (d, 1H), 8.37 (d. 1H), 8.13 (d, 1H), 7.63 (t, 1H), 7.94-7.76 (m, 3H), 7.37 (s, 1H), 6.50 (d, 2H), 6.36 (t, 1H), 3.786 (s, 6H), 2.201 (s, 3H).

Compound **4**: 7.855, 7.824 (d, 2H), 6.728 (s, 1H), 6.630 (d, 2H), 6.54 (d, 2H), 6.399 (t, 1H), 3.764 (s, 6H), 3.284 (t, 4H), 2.197 (s, 3H), 1.57-1.50 (m, 4H), 1.40-1.29 (m, 4H), 0.944 (t, 6H).

Compound **5**: 8.26 (d, 1H), 7.94 (d, 1H), 7.54 (d, 1H), 7.45-7.40 (m, 2H), 7.05 (d, 1H), 6.39 (d, 2H), 6.28 (t, 1H), 5.422, 5.440 (d, 1H), 3.636 (s, 6H), 2.872 (s, 6H), 2.124 (s, 3H).

Compound **6:** 7.993-7.030 (dd, 4H), 6.637 (s, 1H), 6.513 (t, 2H), 6.425 (s, 1H), 3.763 (s, 6H), 2.178 (s, 3H).

Compound **7**: 8.041-7.658 (dd, 4H), 6.432 (t, 1H), 6.567 (d, 2H), 6.694 (s, 1H), 3.765 (s, 6H), 2.218 (s, 3H).

Compound **8**: 8.258-8.046 (dd, 4H), 6.668 (s, 1H), 6.552 (d, 2H), 6.432 (t, 1H), 3.762 (s, 6H), 2.220 (s, 3H).

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Chapter 7

Soluble Polymer- and Solid- Supported Synthesis of Benzoin Esters

Introduction

Photochemically-removable groups have found many applications in biological chemistry[1]. However, all the current photosensitive groups require ultraviolet wavelengths for photolysis, rather than visible or infrared wavelengths that would be less injurious to biological systems and able to penetrate deeper into biological tissues. In nature, under strong selection pressure, biological systems can use visible or far-red photons to accomplish important photochemical reactions very efficiently, such as vision, ion-pumping by bacteriorhodopsin, photosynthesis and phytochrome signaling. In recent years, the need for longer-wavelength caging groups has been supplanted by the advent of two-photon excitation of UV chromephores[6,32,47]. The so-called two-photon excitation is based on the simultaneous absorption of two photons, each having half of the band gap, to excite a fluorophore. A common problem in the application of two-photon uncaging technique is that, in general, cage compounds have very small absorption cross sections for the visible or infrared light used in two-photon excitation. Consequently, the inefficiency of two-photon uncaging greatly limited its applications. Hence, a caging group that can be photochemically removed in high efficiency under the illumination of infrared light via either one-photon or two-photon excitation mechanism is highly desired.

With this in mind, I would like to continue to pursue further research on synthetic chemistry and photochemistry of benzoins. Benzoins are aromatic α-hydroxy ketones of the general formula Ar'CHOHCOAr. Similar compounds containing heterocyclic aromatic components are also classed as benzoins. If the two aromatic components in a

molecule are same, the benzoin is said to be symmetrical; if different, unsymmetrical or "mixed". Benzoins represent a class of molecules with unique photosensitive structures. As reported in literature and as discovered in our own research endeavors, changes on the two aromatic components in benzoin structure may subtly lead to significant change in the photochemical properties[41,42,31,30]. Such a hyper-variable system actually provides an opportunity to achieve the desired properties by finely tuning structures.

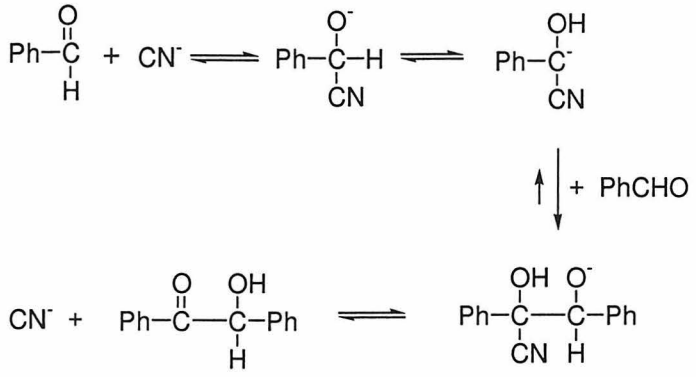
One strategy in this research is to build a large-size library of benzoins. The library will be screened in photolysis experiment for the individual members that possess the desired photochemical properties. In addition to being used as caging groups, benzoins and their derivatives (benzils, enediols, hydrobenzoins) have many other applications. A benzoin library will also facilitate the discovery of those benzoin compounds that are interesting in many other areas. For example, benzoin is a very important synthon for the synthesis of a variety of biologically and pharmaceutically active heterocyclic compounds, such as triazols, tetrazoles, quinolines, pyrroles, oxazoles etc.[44,11,2,19,15,25,26,17] Bezoin and its derivatives are also widely used as metal ligands. Many of the obtained organometallic compounds possess promising pharmacological properties, such as remarkable antitumor, anti-bacterial and antiviral properties[8,29]. Some of these organometallic compounds also have very exciting photochemical properties. For instance, Bis(dithiobenzil) nickel complexes are known to be effective in inhibiting the laser-induced fading of colored thin layers on recording disks, and have recently been applied in optical data storage systems together with near-infrared absorbing cyanine dyes[5]. Benzoins have also been widely used in polymer chemistry and analytical

chemistry. In polymer chemistry, benzoin and benzoin ethers are very efficient radical photoinitiators. In analytical chemistry, benzoin is a highly sensitive and selective fluorescent derivatization reagent for the selective determination of monosubstituted guanidino compounds[18]. It has been used in the selective detection of bioactive peptides containing arginine[4]. Apparently, a benzoin library will be found useful in many of the above areas.

Unsymmetrical benzoins can exist in two isomeric forms differing in the relative positions of the carbinol and carbonyl groups: ArCHOHCOAr′ and ArCOCHOHAr′. In many cases, one isomer is more stable than the other, and the less stable unsymmetrical benzoins can isomerize to the more stable forms. Sometimes, isomeric benzoins of this type yield identical derivatives, since a shift from the less stable to the more stable isomer takes place during the reaction. Therefore, in preparing a library of benzoins, it is critically important to develop a relatively simple, whereas highly efficient and regio-selective synthesis.

A frequently used benzoin synthesis is benzoin condensation[3, 24]. The mechanism of this reaction has been firmly established[22] as shown in Scheme 7.1. Cyanide ion serves a catalytic role in the generation of a cyanohydrin carbanion, which is synthetically equivalent to the benzoyl anion. Since the discovery of the benzoin condensation, both the versatility of the reaction and the yield of symmetrical and unsymmetrical benzoins have been improved[45,12]. However, in the condensation of two different aldehydes, in principle, four benzoins can be produced. It is very time-consuming to purify each compound from the crude reaction mixture. When the two

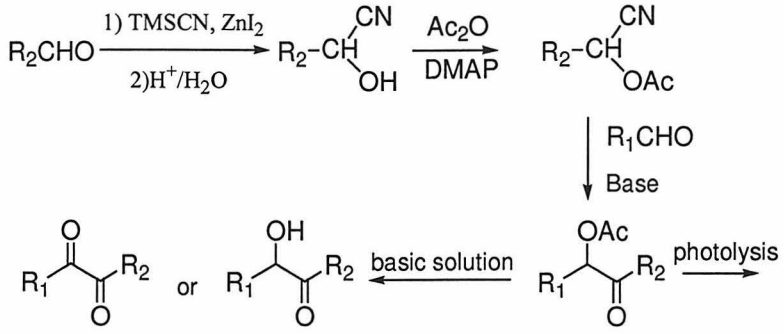
aldehydes are of widely different character, only the more stable form of the two isomeric mixed benzoins is separable due to the reversibility of the reaction and the relative stability of the carbonyl groups in the possible products.



Scheme 7.1. The Mechanism of Benzoin Condensation

One approach for the synthesis of the thermodynamically less stable mixed benzoins involves the addition of an excess of Grignard reagent to a cyanohydrin or to a protected cyanohydrin of an aromatic aldehyde[7,43,21]. Our laboratory also developed a novel synthetic approach in which phenyl dithiane anion formed under *n*-butyllithium treatment reacts with an aromatic aldehyde to generate dithiane-protected benzoins and the subsequent dithiane hydrolysis produces benzoins[46]. However, all of these syntheses use orgnometallic reagents and require very elaborate experimental manipulations. Obviously they are not appropriate approaches for the combinatorial library synthesis. In addition, the use of organometallic reagents also precludes the formation of many benzoins with certain functional groups, such as halide, nitro, etc. In order to prepare a

regio-selective benzoin library, a synthetic approach that involves the generation of a "masked" acyl carbanion[16,13,20,33] was developed (Scheme 7.2). The product, a benzoin ester, can be used directly for photolysis study. The ester bond can also be hydrolyzed to yield benzoins or benzils or other derivatives.



Scheme 7.2. Regio-selective Synthesis of Benzoin Acetates

To prepare a combinatorial library, soluble polymer- or solid- supported synthesis is normally employed because they provide many advantages compared with conventional solution phase synthesis, such as the ease of separation and purification, the convenience of adding excess reagent to move chemical equilibrium or accelerate reactions and so on. The synthetic scheme described in Scheme 7.2 was applied in the soluble polymer- and solid-supported synthesis of benzoin esters. The synthesis developed herein established the protocol for the preparation of combinatorial libraries for benzoins.

Results and Discussion

Poly(ethylene glycol) supported liquid phase synthesis of benzoins

Various approaches have been developed for the synthesis of cyanohydrins. However, in many of these approaches, the formation of cyanohydrins is generally considered to be an equilibrium reaction and therefore could not afford to high yield of cyanohydrins[23]. The two-step synthesis as shown in Scheme 7.2 was selected because it is a general method for the synthesis of various cyanohydrins[9]. Compared with other methods, it gives a nearly quantitative yield and tolerates many functional groups.

Initially, a phase-transfer reaction was used to form benzoin esters. The bi-phasic solvents are benzene/50% NaOH aqueous solution. The phase transfer reagent is triethylbenzylammonium chloride (TEBA). Before the reaction was carried out in a polymer-supported fashion, different starting materials were used to test the feasibility and the yield of this phase-transfer reaction. These pilot reactions resulted in reasonably good regio-selectivity and yield in the range of 40-90% (Figure 7.1).

Since it is a phase-transfer reaction, a liquid-phase preparation is needed for the polymer-supported library synthesis. Poly(ethylene glycol) (PEG) was chosen as the polymer support because many successful applications of the liquid-phase library preparation were resulted from the use of poly(ethylene glycol) as the polymeric support[10]. This linear homopolymer exhibits solubility in a wide range of organic solvents and water. PEG is insoluble in hexane, diethyl ether and *tert*-butyl methyl ether, and these solvents can be used to induce PEG precipitation. Careful precipitation conditions or cooling of polymer solutions in ethanol or methanol yield crystalline PEG

due to the helical structure of the polymer that produces a strong propensity to crystallize[14].

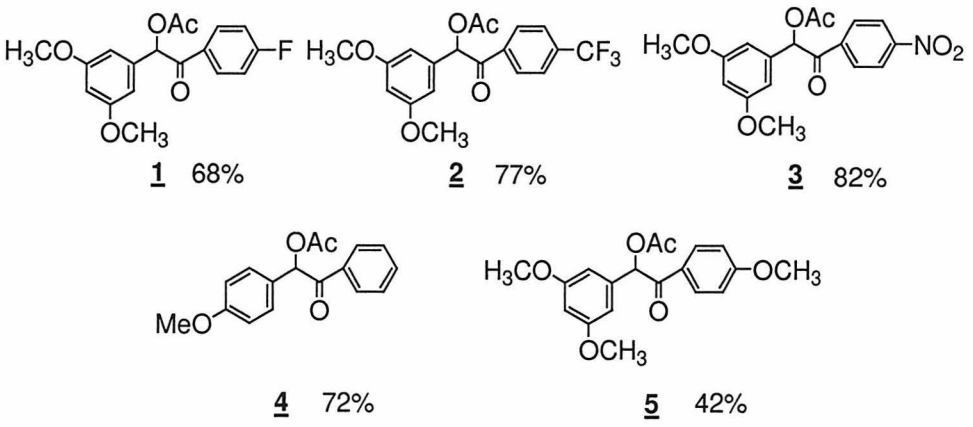
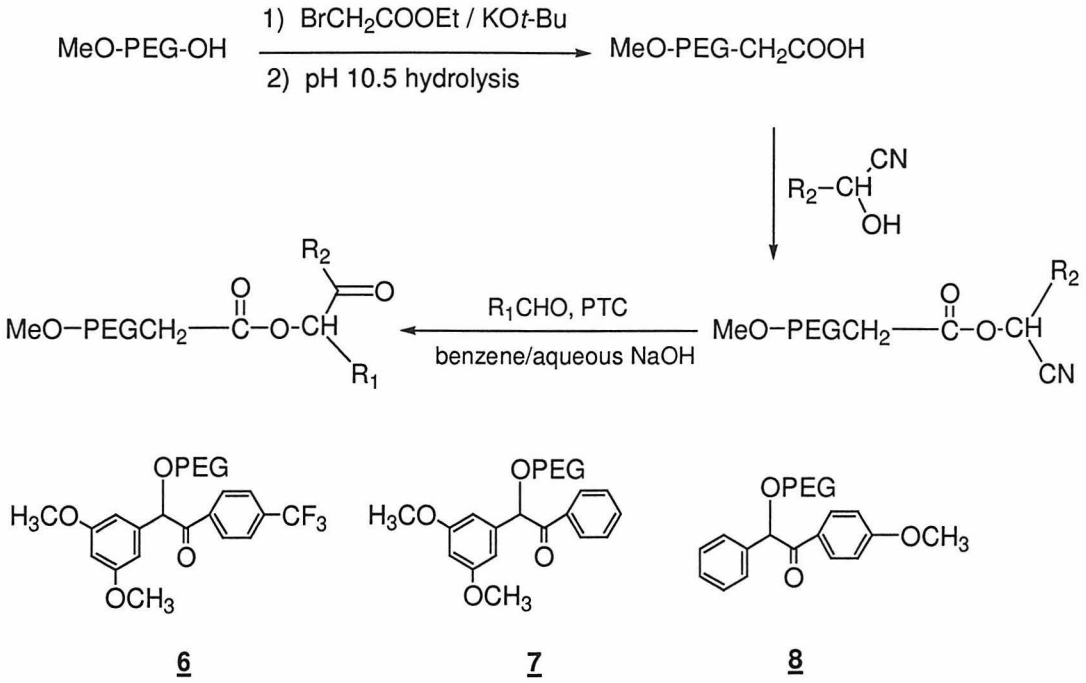


Figure 7.1. Products of Test Reactions for Scheme 7.2

The synthetic procedures in the PEG–supported synthesis of benzoins are illustrated in Scheme 7.3. The product is a benzoin "caged" polymer in which the benzoin is linked with the PEG via an ester bond. A library generated from this method can be used directly for photolysis screening. Upon photolysis, if the benzoin ester synthesized can photolytically cyclize, the formed benzofuran will be released from the polymeric support.



6 **7** **8**

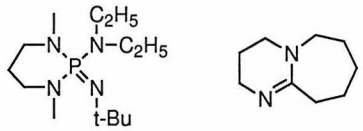
Scheme 7.3. PEG Supported Liquid Phase Synthesis of Benzoins

The PEG supported benzoin synthesis was tested and found to yield benzoin esters **6**, **7**, **8** at very high purity (> 96%). Separation and purification at each step were achieved by precipitation (crystallization) and filtration. Compared with conventional solution phase synthesis, manipulation and purification were much simplified. The synthetic protocol and the methods developed here provide the groundwork for the large-size library synthesis in the future.

Solid supported synthesis of benzoins

Although the above-described liquid-phase polymer-supported synthesis was very successful, a solid-state synthesis, if possible, would be preferred since no yield-losing manipulations, such as precipitation and crystallization, will be needed. However, to use solid-support synthesis, a non-PTC (phase transfer condition) reaction has to be developed to produce benzoin esters. Towards this end, a novel synthesis utilizing non-ionic organic base DBU was developed to replace the phase transfer reaction in producing benzoin esters.

Non-ionic nitrogen bases are widely used reagents or catalysis. However, most of the well-known organic bases are not strong enough to pull off the proton from the acyl cyanohydrin. In recent years, Schwesinger and et al. have developed a series of strong non-ionic organic bases called Schwesinger bases[36,34,37,35,40,38,39]. These bases are tens of thousands of times stronger than conventional organic bases, such as triethylamine. In applications, they have been used in the synthesis of unnatural peptide to achieve peptide backbone alkylation[28,27].

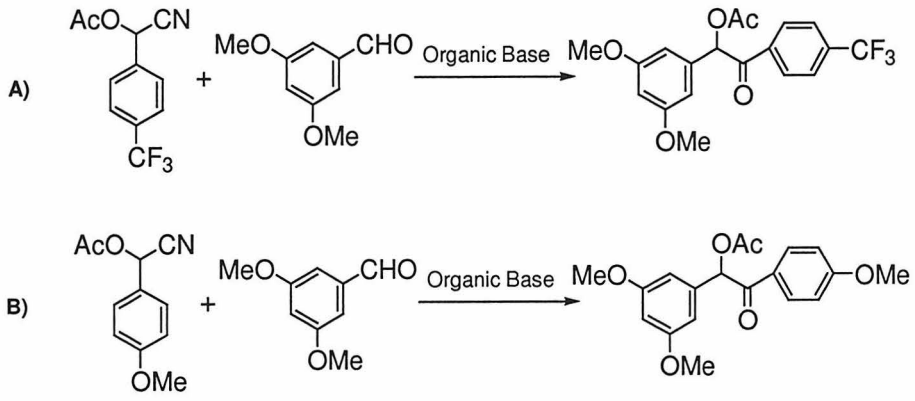


BEMP **DBU**

Figure 7.2. Non-ionic Organic Bases

A commercially supplied Schwesinger base, BEMP, was tested in reaction A (Scheme 7.4) first. The reaction was carried out in acetonitrile and yielded a very dark red color upon addition of BEMP. Purification and characterization indicated that the reaction resulted in a messier mixture compared with what was observed in phase transfer

reactions. More than one fluorescent spot were observed on TLC under the illumination of a UV lamp. The product was separated and the yield was found lower than that from the phase transfer reaction. Although shorter reaction time led to slight improvement on the yield of the product, it is not as good as expected. In test reaction B, it afforded a similar result. It seems that BEMP is a too strong base and may be involved in base catalyzed benzoin isomerizations.

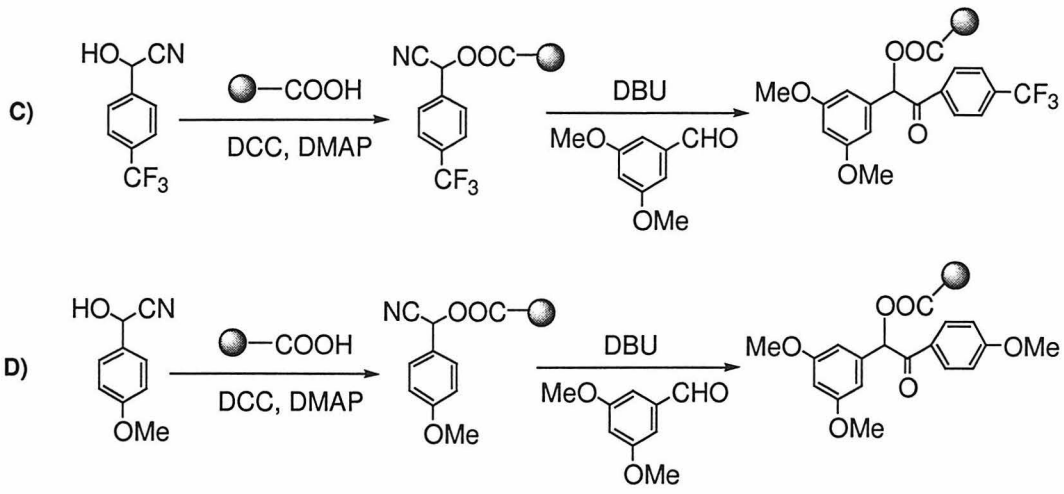


Scheme 7.4. Test Reactions for the Benzoin Acetate Synthesis by Organic Base

DBU, a non-ionic organic base weaker than BEMP, was tested in the benzoin ester formation reactions **A** and **B**. In THF solution, both of the two reactions yielded cleaner product mixtures than those in the phase transfer reactions. Yield of the benzoin esters was also consistently higher than that obtained under the other two reaction conditions.

The two reactions were performed in the solid beads, NovaSyn® TG carboxy resin, purchased from Novabiochem. The NovaSyn® TG carboxy resin is a PEG

polystyrene based resin with 0.2-0.3mmol/g loading capacity. The synthesis led to targeted products (Scheme 7.5) at very high purity.

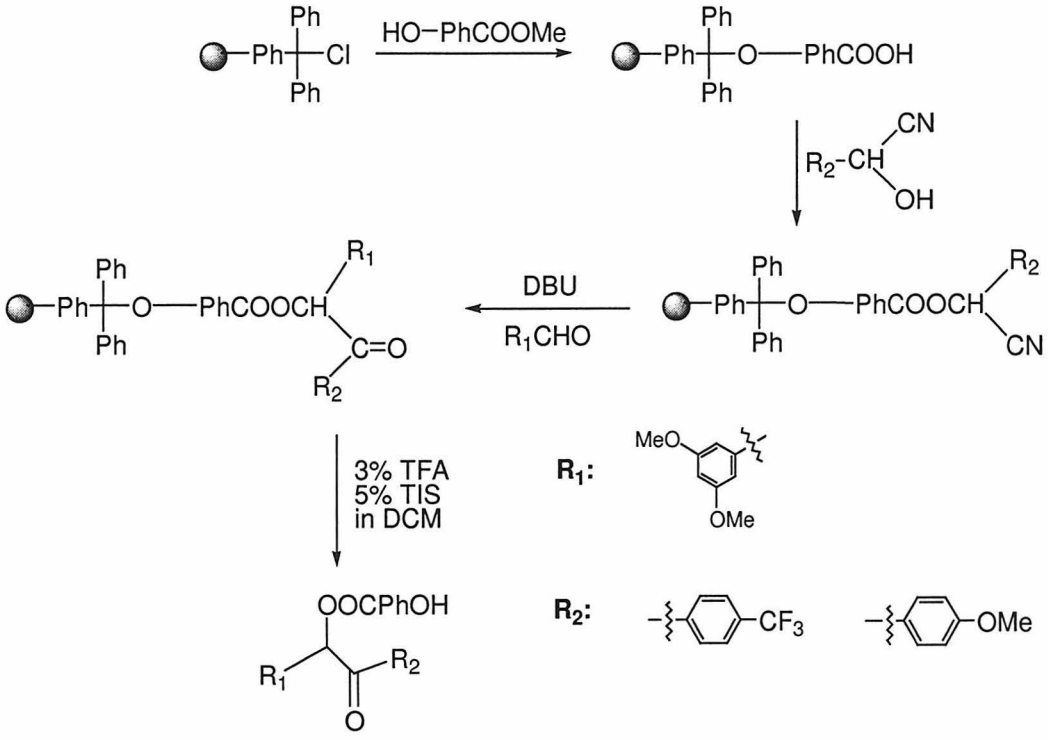


Scheme 7.5. Carboxy Resin Supported Benzoin Synthesis

There are many advantages of using solid resin as a support in organic synthesis, particularly, the ease of separation and purification. However, although direct photolysis can be performed with the resin attached benzoin esters, a quantitative study on the photolysis will be difficult due to the lack of information on the precise amount of attached benzoins. To resolve this problem, a new synthetic scheme based on trityl chloride resin was developed (Scheme 7.6). Such a synthetic scheme allows the release of free benzoin esters by cleaving the trityl ether bond under very mild reaction conditions. As observed in other polymer- and solid-supported syntheses, such a synthetic scheme yielded targeted compounds in very high purity.

Summary

Simple and highly efficient synthesis schemes were developed for the regio-selective synthesis of benzoin esters. The new synthetic approaches utilized a "masked" acyl carbanion to react with aromatic aldehydes in a phase-transfer reaction or under the



Scheme 7.6. Trityl Chloride Resin Supported Benzoin Ester Synthesis

action of DBU, a non-ionic organic base. These newly developed synthesis methods were successfully applied in soluble poly(ethylene glycol) (PEG) supported liquid-phase

synthesis and solid resin supported benzoin ester synthesis. In addition, a synthetic scheme that allows the release of free benzoin esters was also developed using the trityl chloride resin so that precise quantitative characterization of the photolysis of synthesized benzoin esters can be performed. This study established the synthetic protocol for the preparation of large-size libraries of benzoin esters and other benzoin derivatives. It will facilitate further investigations on the photolysis mechanism of benzoins and the design of novel photochemically removable caging groups.

Materials and Methods

General: Anhydrous THF was prepared by refluxing over sodium metal and benzophenone. The trityl chloride and carboxy resins were purchased from Novabiochem. MeO-PEG-5000 was purchased from Aldrich. Other reagents and anhydrous acetonitrile, methylene chloride and pyridine were purchased from Fluka. ^{1}H NMR spectra were recorded on a GE QE300 spectrometer operating at a nominal frequency of 300 MHz. The optical absorption was monitored with a HP 8452 diode array spectrophotometer. HPLC analysis was performed using a Shimadzu LC-6A system equipped with a Vydac C-18 reverse-phase column. Steady-state photolysis was performed with an Oriel 66011 Hg vapor arc lamp operating at 450 W and filtered through water-cooled Schott glass type UG11 and WG320 filters.

Synthesis of compounds 1 – 5 via phase transfer reactions

Preparation of Cyanohydrins. 1.0 equivalent of aldehyde in dichloromethane was allowed to react with 1.1 equivalents of trimethylsilyl cyanide in the presence of a catalytic amount of zinc iodide at room temperature for 24 hour. The solvent was removed under reduced pressure and the residue was added to 1.0N hydrochloric acid at room temperature for 0.5-3.0 hour (TLC was used to monitor the reaction). The solution was extracted with diethyl ether. The ether solution was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The product was purified by silica gel flash column chromatography.

For Compound **1**: Yield 98%. ^{1}H NMR ($CDCl_3$) δ 7.53 (d, 2H), 7.09 (d, 2H), 5.41 (s, 1H), 4.48 (bs, 1H).

For Compound **2**: Yield 97%. ^{1}H NMR ($CDCl_3$) δ 7.75 (d, 2H), 7.65 (d, 2H), 5.46 (s, 1H), 4.5 (bs, 1H)

For Compound **3**: Yield 94%. ^{1}H NMR ($CDCl_3$) δ 8.34 (d, 2H), 7.87 (d, 2H), 6.034 (s, 1H), 3.18 (bs, 1H).

For Compound **4**: Yield 99%. ^{1}H NMR ($CDCl_3$) δ 7.5 (m, 5H), 5.48 (s, 1H), 4.46 (bs, 1H).

For Compound **5**: Yield 97%. ^{1}H NMR ($CDCl_3$) δ 7.46 (d, 2H), 6.95 (d, 2H), 5.498 (s, 1H), 3.835 (s, 3H), 2.172 (s, 3H).

Preparation of Cyanohydrin Acetates. 1.0 equivalent of the cyanohydrin was dissolved in anhydrous dichloromethane. To the solution, 4.0 equivalents of acetic anhydride was

added, followed by addition of catalytic amount of DMAP. The mixture was stirred overnight, quenched with dilute hydrochloric acid, extracted with dichloromethane. The organic layers were collected, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was purified with silica gel flash column chromatography using 4:1 hexanes/ethyl acetate. All the reactions gave quantitative yields.

For Compound **1**: ^{1}H NMR ($CDCl_3$) δ 7.534-7.090 (dd, 4H), 6.375 (s, 1H), 2.140 (s, 3H).

For Compound **2**: ^{1}H NMR ($CDCl_3$) δ 7.748-7.647 (dd, 4H), 6.468 (s, 1H), 2.198 (s, 3H).

For Compound **3**: ^{1}H NMR ($CDCl_3$) δ 8.338-7.715 (dd, 4H), 6.505 (s, 1H), 2.219 (s, 3H).

For Compound **4**: ^{1}H NMR ($CDCl_3$) δ 7.56–7.53 (m, 2H), 7.50-7.42 (m, 3H), 6.412 (s, 1H), 2.167 (s, 3H).

For Compound **5**: ^{1}H NMR ($CDCl_3$) δ 7.45 (d, 2H), 6.96 (d, 2H), 6.354 (s, 1H), 3.834 (s, 3H), 2.143 (s, 3H).

Phase Transfer Reaction of Benzoin Condensation. A two-phase system composed of cyanohydrin acetate (1mmol) in benzene (5mL), 50% sodium hydroxide aqueous solution (0.4mL) and triethylbenzylammonium chloride (TEBA, 15mg) was stirred for 10 minutes and then cooled to 0°C in an ice bath. 3', 5'-Dimethoxybenzaldehyde (for compound **4**, it is *p*-methoxybenzaldehyde) (1mmol) in benzene (5mL) was added at 0°C and stirred for another 0.5-3h monitored by TLC. Water (15mL) was added and the mixture was extracted with diethyl ether. The ether solution was dried over magnesium sulfate and concentrated *in vacuo*. The product was purified with silica gel flash column chromatography using 4:1 hexanes/ethyl acetate.

Compound **1**: Yield 68%. For ^{1}H NMR, please refer to chapter 6.

Compound **2**: Yield 77%. For ^{1}H NMR, please refer to chapter 6.

Compound **3**: Yield 82%. For ^{1}H NMR, please refer to chapter 6.

Compound **4**: Yield 72%. ^{1}H NMR ($CDCl_3$) δ 7.92 (d, 2H), 7.27 (m, 2H), 7.1-6.9 (m, 2H), 3.76 (s, 3H), 2.10 (s, 3H).

Compound **5**: Yield 42%. ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 2H), 6.89 (d, 2H), 6.75 (s, 1H), 6.64 (d, 2H), 6.40 (t, 1H), 3.85 (s, 3H), 3.75 (s, 6H), 2.08 (s, 3H).

PEG Supported Liquid Phase Benzoin Ester Synthesis

Preparation of Carboxymethyl-Poly(ethylene glycol) Methyl Ether (CM-PEG). Poly (ethylene glycol) methyl ether (28g, Average M_n *ca.* 5,000) and potassium *tert*-butoxide (10g) were dissolved in *tert*-butyl alcohol (150mL) by warming to 40°C. Ethyl bromoacetate (5mL) was added over a period of 10 minutes. After the mixture was stirred for 2 hour, the solvent was removed under reduced pressure. The residue was dissolved in 100mL of 1M NaOH. After 2 hours at room temperature, the pH of the mixture was adjusted to 2. The polymer was extracted into chloroform. The organic extract was dried with anhydrous magnesium sulfate and the solvent was removed *in vacuo*. The resultant 25g solid was the product as determined by NMR and FT-IR. ^{1}H NMR ($CDCl_3$) δ 4.22 (s, 2H), 3.64 (m, the ethyl protons of the PEG backbone), 3.50 (t, 2H), 3.38 (s, 3H).

Loading Cyanohydrin onto CM-PEG. Cyanohydrin (10mmol) was added to the 50mL anhydrous dichloromethane solution of CM-PEG (5g, 1mmol). To the mixture, DCC (3

mmol) was added, followed by DMAP (3mmol). After 36 hr of stirring at room temperature, diethyl ether was slowly added to the vigorously stirred reaction mixture. The precipitate was collected, thoroughly washed with diethyl ether and dried under vacuum. The product was confirmed with ^{1}H NMR. The chemical shift of the single methoxy group (δ = 3.38 ppm) on CM-PEG was used as the internal standard which was compared with the integration of aromatic protons. The result indicated that the reaction yield the ester linkage quantitatively.

CM-PEG Supported Benzoin Ester Formation via Phase Transfer Reaction. A two-phase system composed of CM-PEG-cyanohydrin acetate (2g) in benzene (20mL), 50% sodium hydroxide aqueous solution (2mL) and triethylbenzylammonium chloride (TEBA, 25mg) was stirred for 10 minutes under argon flow and then cooled to 0°C in an ice bath. 3', 5'-Dimethoxybenzaldehyde (10mmol) in benzene (10mL) was added at 0°C and stirred for another 2hour (FT-IR was used to monitor the disappearance of -CN and formation of carbonyl group). At 0°C, ice-cold 1.0N hydrochloric acid aqueous solution (25mL) was added slowly. The mixture was extracted with chloroform. The organic extract was dried with anhydrous magnesium sulfate and concentrated. Diethyl ether was added and the precipitate was filtered, washed with diethyl ether and dried under vacuum. The purity of the product was determined to be >92% with ^{1}H NMR as described above.

Test Reactions for the Benzoin Acetate Synthesis with DBU (Scheme 7.4)

To a solution of cyanohydrin acetate (1mmol) in THF (15mL), DBU (1.4mmol) was added. The mixture was stirred at room temperature under argon flow for 30 minutes before it was cooled to 0°C in an ice bath. 3', 5'-dimethoxybenzaldehyde (1mmol) was added and the reaction was monitored with TLC. In about 30-60 minutes, the reaction was complete. Saturated ammonium chloride was added to quench the reaction. The mixture was extracted with diethyl ether and the organic extract was dried over anhydrous magnesium sulfate and concentrated. The product was purified by silica gel flash column chromatography using 4:1 hexanes/ethyl acetate. Reaction A gave a yield of 89% and reaction B gave a yield of 72%.

Carboxy Resin Supported Benzoin Synthesis (Scheme 7.5)

Loading Cyanohydrin onto Carboxy Resin. Cyanohydrin (10mmol) was added to the 100mL anhydrous dichloromethane suspension solution of NovaSyn® TG carboxy resin (5g). To the mixture, DCC (10mmol) was added, followed by DMAP (10mmol). After 48 hr of stirring at room temperature, the solid resin was removed from the solution by filtration, washed with dichloromethane thoroughly and dried under vacuum.

Carboxy Resin Supported Benzoin Ester Formation via Phase Transfer Reaction. To the suspension solution of the above prepared resin (2g) in anhydrous THF (50mL), DBU (1.5mmol) was added. The mixture was stirred for 30 minutes at room temperature and then cooled to 0°C. 3', 5'-dimethoxybenzaldehyde (6mmol) was added and the solution

was stirred for 2h. The resin was filtered, washed with THF and dichoromethane and dried under vacuum.

Photolysis of the Benzoin Esters Attached to Solid Resin. The above resin (0.1g) prepared for reaction A was suspended in dichloromethane (2mL) under stirring and illuminated by arc lamp. Upon photolysis the benzofuran will be released from the resin support. UV-Vis spectrometer was used to monitor the benzofuran formation in the solution. TLC indicated that only benzofuran was presented in the solution.

Trityl Chloride Resin Supported Benzoin Ester Synthesis (Scheme 7.6)

Trityl chloride resin (2g, with loading capacity: 0.4-1.0mmol/g) was suspended in anhydrous dichloromethane (40mL). To this suspension, methyl 4-hydroxybenzoate (0.6g, 4mmol) dichloromethane solution (10mL) was added followed by the addition of pyridine (4mmol). The mixture was stirred for overnight at room temperature. The resin was filtered, washed with dichloromethane and dried under vacuum.

The resin obtained from the above preparation was treated with 100mL of 1.0N NaOH aqueous solution for 2 hours at room temperature. The resin was filtered and added into 100mL of 0.01N hydrochloric acid solution. The resin was filtered again and washed with 0.01N hydrochloric acid, double-distilled water, lyophilized and further dried under high vacuum.

Cyanohydrin (4mmol) was added to the anhydrous dichloromethane suspension solution (40mL) of the above prepared resin (1g). To the mixture, DCC (4mmol) was

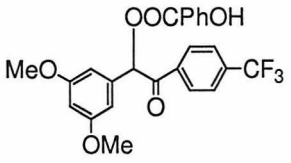
added, followed by DMAP (4mmol). After 48 hr of stirring at room temperature, the solid resin was removed from the solution by filtration, washed thoroughly with dichloromethane, water, methanol and THF and dried under vacuum.

To the suspension solution of the above resin in anhydrous THF (50mL), DBU (1.5mmol) was added. The mixture was stirred for 30 minutes at room temperature and then cooled to 0°C. 3', 5'-dimethoxybenzaldehyde (3mmol) was added and the solution was stirred for 2h. The resin was filtered and washed with THF and dichoromethane and dried under vacuum.

To release the free benzoin ester, the resin was treated with 5% TFA in dichloromathane (10mL). After stirring at room temperature for 2 hour, the resin was filtered and washed with dichloromethane. The liquid was collected and concentrated under reduced pressure. TLC indicated a single compound in the residue.

For **a**: ^{1}H NMR ($CDCl_3$) δ 8.14 (d, 2H), 8.04 (d, 2H), 7.66 (d, 2H), 7.06 (d, 2H), 6.69 (s, 1H), 6.57 (d, 2H), 6.43 (t, 1H), 3.77 (s, 6H), 4.30 (bs, 1H).

For **b**: ^{1}H NMR ($CDCl_3$) δ 8.13 (d, 2H), 7.99 (d, 2H), 7.08 (d, 2H), 6. 89(d, 2H), 6.75 (s, 1H), 6.64 (d, 2H), 6.40 (t, 1H), 3.85 (s, 3H), 3.75 (s, 6H), 4.40 (bs, 1H).



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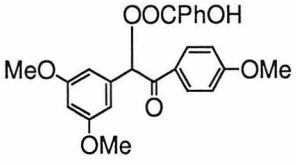

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