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The preparation and reactivity of biologically important p-benzoquinol imines. In search of the "ultimate" carcinogen of N-acetyl-2-aminofluorene

Clark, William M., Jr., Ph.D.
The Ohio State University, 1992
THE PREPARATION AND REACTIVITY OF BIOLOGICALLY IMPORTANT
\p-bENZOQUINOL IMINES. IN SEARCH OF THE "ULTIMATE"
CARCINOGEN OF N-ACETYL-2-AMINOFLUORENE.

Dissertation

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

William M. Clark, Jr.

****

The Ohio State University
1992

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the Incorporation of Sulfur Nucleophiles during Metabolism of Acylated Amines. Clark, W.M.;

Detection of Intermediates Formed during the Hydrolysis of N-(Sulfonatoxy)-
N-Acetyl-4-Aminobiphenyl and N-Benzoyl-4-Hydroxy- 4-Phenyl-2,5-Cyclohexadienone Imine.
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FIELDS OF STUDY

Major Field: Chemistry

Studies in -

Anodic Oxidation Methodology for the Preparation of
Synthetically Useful Organic Compounds

Pharmacological Aspects of Glutathione in Drug Metabolism
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Chapter I

Hepatic-Induced Carcinogenesis of \(N\)-Acylated Anilines

Discussion

Over the years an intense investigative effort has developed a remarkable understanding of the metabolism of exogenous compounds in the family of chemotherapeutics, pesticides, and other xenobiotics. The toxicity observed for most of these agents is generally not associated with the parent compound itself but rather an hepatic-induced intermediate capable of binding with cellular macromolecules. Much of the effort has focused on elucidating the structure of these chemically reactive metabolites by isolating and characterizing their nucleic acid and glutathione-conjugates. Although a great deal of information has been obtained with this approach, this subject remains an intense area of research due to the lack of understanding of the chemistry of many of these important intermediates.

The metabolic activation of the once widely used nonprescription drug, phenacetin, first introduced in 1898 for its analgesic and antipyretic effects and available in some countries up to 1986, has particularly attracted an intense amount of interest. While relatively safe when administered in small doses, overdose of the drug...
led to serious damage in human and animal subjects. For example, phenacetin (1) was shown to reduce the lifetimes of erythrocytes, cause anemia, and methaemoglobinaemia. Cases of kidney necrosis, kidney carcinomas, bladder cancer, and tumors in the urinary system were also reported, as well as the demonstration of the carcinogenicity of 1 in laboratory animals. For example, when fed a diet containing 2.5% of 1, male and female Sprague-Dawley rats showed neoplasms in 91% and 77% of the test animals respectively.

Investigations have developed two important paths (Scheme I) concerning the metabolic activation of 1 in liver tissue. The first pathway begins with initial oxidative de-ethylation to form acetamin-
ophen 2, followed by the generation of the electrophilic intermediate, \( N\)-acetyl-\( p\)-benzoquinone imine (NAPQI) (3), via microsomal action of the cytochrome P-450 mixed oxidase system.\(^{16}\) In support of this metabolic route, in vivo,\(^{17}\) and in vitro\(^{18}\) studies have shown NAPQI to be highly cytotoxic, capable of arylating protein sulfhydryl groups,\(^{19}\) as well as the tripeptide glutathione (\( \gamma \)-L-glutamyl-L-cysteinylglycine), yielding the covalently bound thioether adducts such as 4, isolated from the urine of rats.\(^{1a}\) In addition, in vivo labeling studies\(^{20}\) using \([p-^{18}O]\)-phenacetin showed approximately 90% of the \(^{18}O\) label in 4 was retained, indicating the top route in Scheme 1 may in fact be a major metabolic path. However, the cytotoxic effects of acetaminophen do not fully account for the toxicity observed for phenacetin.\(^{21}\)

The alternative route begins with \( N\)-hydroxylation to give \( N\)-hydroxyphenacetin 5, a metabolite found in the urine of test animals dosed with phenacetin.\(^{22}\) This metabolite may then form a conjugate with glucuronic acid catalyzed by liver enzymes\(^{23}\) to yield the highly reactive intermediate 6. This species is then believed to decompose to the nitrenium ion-solvent separated ion pair and react with water at a diffusion-controlled rate to form 7. Decomposition of the transient intermediate 7, the ethyl hemi-ketal of \( N\)-acetyl-\( p\)-benzoquinone imine, will then yield the cytotoxic NAPQI. Labeling studies in fact provide strong evidence for the latter part of this mechanism.\(^{20}\) Incubation of \( N\)-hydroxyphenacetin glucuronide in the presence of glutathione and \( H_2^{18}O\), which was 22% \(^{18}O\), gave the
acetaminophen-glutathione conjugate 4 with 24% of the phenolic oxygen as $^{18}O$. Thus all of the oxygen of the ethoxy group was replaced by oxygen from water.

With respect to the two metabolic routes above, the bottom path of Scheme I best accounts for the adverse effects on the peripheral organs of the liver. Stability and toxicity must play a role on the nature of the proposed intermediate, and in effect the higher reactivity of NAPQI may account for much of the liver damage associated with the overdose of 1. However, the lower reactivity of $N$-hydroxyphenacetin would allow clearance from the liver and explain the necrosis and carcinoma observed in the kidney and other organs of the body.

In addition to phenacetin, numerous other aromatic amines exhibit carcinogenic behavior and have been the focus of much investigation. In particular, $N$-acetyl-2-aminofluorene (2-AAF) 8 has been a widely studied model carcinogen in this class of compounds. Initially patented as an insecticide in 1940, 2-AAF was never marketed due to its high toxicity in test animals. As observed with phenacetin, enzymatic hydroxylation appears to be the important step in the metabolic activation sequence. Shown in Scheme II, a number of ring-hydroxylated metabolites (excreted as glucuronide and sulfate conjugates) have been isolated and characterized from the urine of rabbits and rats. In addition glutathione-adducts 10 and 11 have been isolated from the urine of rats as well as 2-[$N$-(deoxyguanosin-8-yl)amino]fluorene 12 from the liver and kidney.
of rats$^{27a}$ and bladder and liver of dogs$^{27b}$ upon DNA cleavage (Scheme III).

With respect to toxicity, the ring-hydroxylated metabolites did not exhibit carcinogenicity, while the $N$-hydroxy-2-aminofluorene ($N$-hydroxy-AAF) 9 proved to be a more active carcinogen than the parent amide.\textsuperscript{28} For example, in vivo studies have shown $N$-hydroxy-AAF exhibits a higher level of binding to cellular macromolecules relative to 2-AAF. However when model studies were undertaken in vitro, low levels of reactivity of $N$-hydroxy-AAF with proteins and nucleic acids
were observed. Thus a second activation step has been postulated to account for the covalent binding of 2-AAF residues in vivo. This second activation step has been at the core of much investigation and debate.

The 2-aminofluorene (AF) moieties such as 12 appear to be confined to nucleic acid adducts and are thought to originate from

Scheme III. Glutathione- and Guanosine-Conjugated Metabolites of N-Acetyl-2-Aminofluorene.

either N-acetoxy-2-aminofluorene (N-acetoxy-AF) 14 or from the deacylation of 9 to give the hydroxylamine (N-OH-AF) intermediate 13 both of which are highly reactive (Scheme IV). Enzymatic deacylation of N-hydroxy-AAF to give the unstable hydroxylamine has been reported and non-enzymatic acid-catalyzed binding with guanine in nucleic acids was demonstrated. However reactivity at neutral pH appeared to be
low. Therefore a considerable amount of attention has focused on understanding the reactivity of the \( N \)-acetoxy-AF intermediate \( 14 \). This metabolite can be generated via transacylation of \( N \)-hydroxy-AF by action of \( N \)-arylhydroxamic acid \( N,O \)-acyltransferase (AHAT), an enzyme present in numerous mammalian tissues.\(^{31}\) Unfortunately \( N \)-acetoxy-AF is so reactive, inactivation of the AHAT enzyme itself is observed, hindering attempts to study this important intermediate. In addition all efforts to isolate or synthesize this putative species have proven to be very difficult.\(^{32}\) However, recent success in the in situ preparation of \( 14 \) in the presence of deoxyguanosine (dG), giving \( 12 \) (14%) as the sole isolated product (Scheme V), provides strong evidence that \( 14 \) indeed may be an important intermediate in the metabolic activation of 2-AAF.\(^{33}\) In addition, calculation of kinetic rate data of a derivative of \( 14 \) fit a second-order plot, suggesting this reaction follows an \( S_N^2 \) mechanism.\(^{34}\)
This metabolic pathway, however, does not explain the formation of the glutathione-conjugates 10 and 11. The first evidence for conversion of N-hydroxy-AAF to an active-ester intermediate came from model studies on the derivative, N-acetoxy-AAF 15. This ester has been shown to react in vitro under physiological conditions with nucleic acids (guanine) and the nucleophilic centers of proteins (methionine, tyrosine, tryptophan, and cysteine). For example, incubation of N-acetoxy-AAF 15 at 37 °C with glutathione in potassium phosphate buffer solution (pH = 7.4) gave the 1-, 3-glutathione conjugates 10 and 11 in 10% and 7% yield respectively (Scheme VI), as well as the 4- and 7-substituted adducts 16 and 17. It is important to note in vivo studies have not detected the glutathione conjugates 16 and 17.

With respect to the biological generation of N-hydroxy-AAF, the non-enzymatic conversion of 9 to N-acetoxy-AAF with acetyl-CoA has been reported. However a high optimal pH (pH = 10.0) and 10% - 20%
acetylation under neutral conditions strongly suggests N-acetoxy-AAF is not the primary metabolic intermediate of N-hydroxy-AAF activation. Similarly the NO-glucuronide of 2-AAF exhibited very low reactivity with nucleophilic proteins in neutral medium. Therefore the most recent investigations have focused on the more reactive ester, e.g., the sulfate ester (AAF-N-sulfate) of N-hydroxy-AAF, present under physiological conditions in the form of its sodium salt (Scheme VII). The activating enzyme, sulfotransferase, is present in liver cytosol and utilizes PAPS, "a specific sulfate donor in the enzymatic sulfation of various hydroxy compounds (e.g. steroids)". A number of experimental investigations provide strong evidence for the existence of this highly reactive intermediate. For example, when 2-AAF and acetaminophen (a sulfate scavenger) were simultaneously administered, only low levels of 2-AAF protein-bound metabolites were
detected unless sulfate was also administered to regenerate the sulfate pool.\textsuperscript{24}

**Scheme VII. Enzymatic Activation of N-Hydroxy-AAF.**

![Diagram of Scheme VII]

Also, administration of sulfate increased the amount of 2-AAF protein-bound residues while other anions did not, and pretreatment of male Wistar rats with the sulfotransferase inhibitor pentachlorophenol reduced excretions by 50\%\textsuperscript{26} In addition, further evidence was

**Scheme VIII. Decomposition Products of AAF-N-Sulfate in 5 Vol % CH\textsubscript{3}CN-H\textsubscript{2}O Potassium Phosphate Buffer Solutions.**

![Diagram of Scheme VIII]
provided with a comparative study of the carcinogenicity of 2-AAF in female Sprague-Dawley and Fischer rats. Little carcinogenic behavior was observed in the female Fischer rats, whereas 2-AAF induced a high incidence of mammary tumors in the former test strain. This was correlated with the lower sulfotransferase activity detected in the female Fisher rat in relation to a substantially higher activity of the enzyme in the female Sprague-Dawley rats.

Due to the high instability and difficulty in purification of AAF-N-sulfate, only a few model studies have been undertaken with this active ester derivative. The most noteworthy of these investigations was the characterization of the breakdown products of AAF-N-sulfate (Scheme VIII) in 5 vol % CH₃CN-H₂O potassium phosphate buffer solutions (pH = 3.6, 4.7, 7.8) at 5 °C by Novak and coworkers. Interestingly, the buffer pH affected the yields of the three major characterized products 21, 23, and 24 (Table 1).

<table>
<thead>
<tr>
<th>pH</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21</td>
</tr>
<tr>
<td>7.8</td>
<td>9</td>
</tr>
<tr>
<td>4.7</td>
<td>65</td>
</tr>
<tr>
<td>3.6</td>
<td>83</td>
</tr>
</tbody>
</table>

Table 1. Effect of pH on Product Yield of AAF-N-Sulfate Decomposition
At pH = 7.8, the major hydrolysis products are 21 (9 %), 23 (11 %), and 24 (51 %) with 23 stable to the reaction conditions, i.e., 24 is not formed by its hydrolysis. With lower pH the yields dramatically change: pH = 4.7, 21 (65 %), 24 (6 %) and pH = 3.6, 21 (83 %) and 24 (1 %). Apparently, "24 is formed by the decomposition of 22 at pH = 7.8, and 21 formed more rapidly from the decomposition of 20, while the product yield variation with pH indicates an acid catalyzed reversion of 22 to the nitrenium ion 20".40

The decomposition of the active ester 19 to the intermediate 23 is not without precedent. Gassman has shown when the methanesulfonate ester of N-hydroxyacetanilide 26 was warmed to -55 °C in non-nucleophilic solvents (Scheme IX), rearrangement of the sulfonate

Scheme IX. Decomposition Products of Methane Sulfonate Ester of N-Hydroxyacetanilide.
ester to the ortho position of the aryl moiety gave 29 in quantitative yield. However, when a -78 °C solution of 26 was added to refluxing methanol, a mixture containing 27 (52 %), 28 (4 %) and 29 (19%) was characterized. In addition, application of kinetic rate data of the internal return reaction of derivatives of 26 to the Hammett $\sigma^+\rho$ equation gave $\rho = -9.24$. This large negative $\rho$ value undoubtably corresponds to a heterolytic N-O bond cleavage of the sulfonate ester. Indeed a Hammett $\sigma^+$ plot vs the rate constant of decomposition of 19 to form 20 in Scheme VIII (pH and buffer independent) gave a $\rho = -5.7$ ($r = 0.97$), strongly supporting the above mechanism. It is therefore apparent the intermediate 22 may play a role in the biological effects attributed to AAF-N-sulfate 19. This importance is emphasized with the reported stability of species 22, e.g., half-life ca. 2.0 min at 37 °C and pH = 7.0, a long-lived intermediate which may be capable of transporting to extrahepatic organs and tissue. However, to date, attempts to synthesize or isolate 22 have not been reported and therefore the chemistry of this metabolite is poorly understood.
List of References


19. See references 12 - 17 in 4c.


21. Extrahepatic toxicities associated with the overdose of phenacetin have not been reported for acetaminophen.


24. For an excellent review on the toxicity of N-acetyl-2-aminofluorene and other aromatic amines, see: Kriek, E. *Biochemica et Biophysica Acta* 1974, 355, 177.


Chapter II

The Anodic Oxidation of 4-Alkyl- and 4-Aryl-N-Acylated Anilines.
The Preparation of Biologically Important p-Benzoquinol Imines.

Introduction

This introduction will describe a general preparative route to a new class of synthetically useful compounds, e.g., \( N \)-acylated-p-benzoquinone imine ketals, recently developed by our research group. In addition it was designed to provide the reader unfamiliar with this important new area of research, a brief explanation of some of the experimental conditions for a more complete understanding of the chemistry. With regards to this doctoral thesis, the main objective is to expand this methodology into the p-alkyl- and p-aryl- class of anilides and ultimately to synthesize and study the biologically important p-benzoquinol imine discussed in chapter 1.

The appreciation of organic electrochemistry to effect useful chemical transformations has grown rapidly in recent years culminating with the publication of numerous books and review articles. Indeed a wide variety of organic electrochemical transformations can be carried out rapidly and in good yield, using relatively simple equipment (see Figure 1), performed under mild conditions with the added advantage of avoiding hazardous reagents or by-products. In
addition, not only can electrochemistry improve upon numerous chemical processes, many reactions can only be carried out via electrochemical transformations.

The anodic oxidation of electron-rich aromatic systems is an area of organic electrochemistry which best typifies the utility of electrochemical transformations. For example, anodic oxidation of 1,4-dimethoxybenzene in methanolic potassium hydroxide affords the benzoquinone bisketal in high yield, a compound with numerous synthetic applications and unavailable via traditional chemical methodology.\textsuperscript{46} By analogy, the anodic oxidation of the corresponding anilides, however, have received much less attention, primarily due to the complex nature of the product mixtures reported in previous investigations (Scheme X).\textsuperscript{47a-c}
Recently from these laboratories, the first electrochemical conversion of 4-methoxybenzanilide to N-acylated p-benzoquinone imine ketal derivatives was reported in methanolic solvent (Table 2). For example, anodic oxidation of 4-methoxybenzanilide 30a in 5% H₂O:CH₃OH solution using 2% LiClO₄ as electrolyte and NaHCO₃ as base gave N-benzoyl-p-benzoquinone imine dimethyl ketal 32a in 92% yield. The anodic oxidations in Table 2 were performed under constant current conditions in a single-cell electrochemical apparatus using a Pt anode and cathode.

The successful anodic oxidation of the N-acylated anilides shown below is a function of a number of experimental parameters. For
Table 2: Anodic Oxidations of Anilides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compd</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>R&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Current Efficiency (%)</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30a</td>
<td>Ph</td>
<td>H</td>
<td>H</td>
<td>60.90</td>
<td>92.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>30b</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>H</td>
<td>-.87</td>
<td>89.79&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>30c</td>
<td>Ph</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>30d</td>
<td>OBu&lt;sup&gt;e&lt;/sup&gt;</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>55</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td>30e</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>98</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>30f</td>
<td>Ph</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;Et</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>91</td>
<td>97</td>
</tr>
<tr>
<td>7</td>
<td>30g</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;Et</td>
<td>H</td>
<td>94</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>30h</td>
<td>Ph</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;Et</td>
<td>H</td>
<td>95</td>
<td>94</td>
</tr>
<tr>
<td>9</td>
<td>30j</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;Cl</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>64</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>30l</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;Cl</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>64</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>30m</td>
<td>OBu&lt;sup&gt;e&lt;/sup&gt;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;Cl</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>81</td>
<td>73</td>
</tr>
<tr>
<td>12</td>
<td>30n</td>
<td>OBu&lt;sup&gt;e&lt;/sup&gt;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;F</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>87</td>
<td>86</td>
</tr>
</tbody>
</table>

<sup>a</sup>Yield of material of > 95% purity by <sup>1</sup>H NMR spectroscopy from 0.1-1.0 g oxidation isolated by chromatography or crystallization.

<sup>b</sup>Yield of crystalline material from a 10 g oxidation.

<sup>c</sup>Yield of distilled material from a 5 g oxidation.
Scheme XI. Anodic Oxidation of 4-Methoxybenzanilide 30a without Base.

\[
\begin{align*}
\text{H-N-CPh} & \quad \xrightarrow{2\% \text{ LiClO}_4} \quad \text{CH}_3\text{O} \quad \text{NH-CPh} \\
\text{O} \quad \text{30a} & \quad \xrightarrow{\text{THF}} \quad \text{NaH} \quad (1.0 \text{ eq}) \quad \text{31a} \\
\text{O} \quad (92\%) & \quad \xrightarrow{} \quad \text{H}_3\text{CO} \quad \text{OCH}_3 \quad \text{32a} \quad (88\%)
\end{align*}
\]

example, performing the anodic oxidation with a copper cathode resulted in incomplete oxidation of the starting anilide 30 presumably due to reduction of the product at the cathode surface.

Table 3. Effects of Current upon the Anodic Oxidation of 30b Using NaHCO₃ as Base.⁴⁹

<table>
<thead>
<tr>
<th>NaHCO₃ (g)</th>
<th>30b (g)</th>
<th>Solvent (mL)</th>
<th>Current (A) (min)</th>
<th>Ratio 31b/32b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.1</td>
<td>5% H₂O/CH₃OH (150)</td>
<td>0.1 (20)</td>
<td>&lt; 1:99</td>
</tr>
<tr>
<td>1.0</td>
<td>0.1</td>
<td>5% H₂O/CH₃OH (150)</td>
<td>0.2 (10)</td>
<td>&lt; 1:99</td>
</tr>
<tr>
<td>1.0</td>
<td>0.1</td>
<td>5% H₂O/CH₃OH (150)</td>
<td>0.5 (4)</td>
<td>3:97</td>
</tr>
<tr>
<td>1.0</td>
<td>0.1</td>
<td>5% H₂O/CH₃OH (150)</td>
<td>1.0 (2)</td>
<td>16:84</td>
</tr>
<tr>
<td>1.8</td>
<td>1.0</td>
<td>3% H₂O/CH₃OH (250)</td>
<td>0.1 (194)</td>
<td>5:95</td>
</tr>
<tr>
<td>1.8</td>
<td>1.0</td>
<td>3% H₂O/CH₃OH (250)</td>
<td>0.5 (40)</td>
<td>25:75</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>CH₃OH (100)</td>
<td>0.3 (280)</td>
<td>12:88</td>
</tr>
<tr>
<td>18ᵇ</td>
<td>10.0</td>
<td>CH₃OH (250)</td>
<td>0.4 (685)</td>
<td>44:56</td>
</tr>
</tbody>
</table>

⁻Oxidations conducted with a 0.5 x 3 cm Pt cathode and a 5 cm diam x 5 cm 45 mesh Pt anode using 1.5% LiClO₄ in methanol.
However this effect was not observed for a platinum electrode which has a lower overvoltage for hydrogen and therefore employed in all the anodic oxidations.

Equally important was the addition of excess solid NaHCO$_3$ to the reaction medium. When the anodic oxidation of 30a was not performed in the presence of base, the 1,4-addition product 31a was isolated in 86% yield (Scheme XI). Interestingly, the instability of 31a affords the quinone imine ketal 32a upon standing at room temperature or via the addition of a strong base such as sodium hydride. However, since NaHCO$_3$ is essentially insoluble in methanolic solution, variable amounts of water in the reaction medium gave different yields of products 31 and 32. As shown in Table 3, an anodic oxidation performed in methanol gave higher yields of the 1,4-addition product 31b relative to the optimal reaction solvent of 5% aqueous methanol, indubitably due to the increased solubility of the sodium bicarbonate. In addition, higher current densities increased formation of 31b and demonstrated the anodic oxidations were best performed between 0.1 - 0.2 amperes.

Mechanistically, the anodic oxidation of p-methoxyanilides in methanolic solution can be rationalized via the EEC$_r$C$_p$ mechanism, the favored reaction sequence for the anodic oxidation of 1,4-dimethoxybenzene to p-benzoquinone bis(dimethyl) ketals. The EEC$_r$C$_p$ mechanism is best described in the text below:

The EEC$_r$C$_p$ mechanism involves two electrochemical steps (EE) forming an aromatic radical cation, III-2, and a methoxy radical at the platinum anode. Reaction of the radical cation with the
methoxy radical ($C_r$, the radical combination step) at the electrode surface forms III-3 (Scheme XII). The preference for addition of methoxy radical at C-4 relative to C-2 may be related to the thermodynamic benefit of having two oxygen atoms bonded to the same carbon. This step is followed by either proton removal from III-3 to give III-5 or addition of methanol to III-3 to afford III-4 ($C_p$, the polar step).

Scheme XII. EEC_rC_p Mechanism Applied to Anilide Oxidation

It should also be noted that addition of sodium bicarbonate to a methanolic solution of the 1,4-addition product 31 does not promote
facile elimination of methanol. Apparently the role of the base is to deprotonate the intermediate III-3 (Scheme XII) prior to addition of methanol.
Results

As discussed in the preface, understanding the metabolic activation of the carcinogen, N-acetyl-2-aminofluorene (2-AAF) has been the subject of numerous investigations. From the evidence currently available, the hepatic-induced cytotoxicity of 2-AAF appears to follow a two step biochemical process (Scheme XIII). Investigations to date support initial oxidation of the amide nitrogen giving N-hydroxy-N-acetyl-2-aminofluorene, a relatively stable intermediate which is activated to the more reactive ester derivative, AAF-N-sulfate 19, the species believed to act as the primary mediator of the carcinogenic activity of the parent amide. However, recent

Scheme XIII. Hypothesized Metabolic Path of 2-AAF.

\[
\text{Scheme XIII. Hypothesized Metabolic Path of 2-AAF.}
\]
investigations suggest decomposition of AAF-N-sulfate in water giving the quinol imine intermediate 22, may occur prior to AAF-N-sulfate undergoing reactions with cellular proteins and nucleophiles. The unavailability of 22, however, has hindered further investigations into its biological role as the "ultimate" carcinogenic metabolite.

As discussed in the introduction, our laboratory recently reported a general preparative method for the synthesis of p-benzoquinone imine ketals via anodic oxidation of 4-methoxybenzanilides in methanolic solution. To further investigate the scope and utility of this reaction, the anodic oxidation of p-alkylbenzanilides was examined. In addition, this investigation was undertaken with the hope of eventually applying this methodology towards the synthesis of the p-benzoquinol imine 22.

Initial studies were done with N-benzoyl-p-toluidine 33 to determine the optimum conditions for the anodic oxidations of p-alkyl substituted anilide derivatives. A preliminary anodic oxidation reaction in 5% H2O - CH3OH solution using 2% LiClO4-3H2O as electrolyte gave the 4-methyl-benzoquinol ether imine 34a in 34% yield (Scheme XIV) and attempts to further isolate and characterize the complex mixture of side-products was unsuccessful. However electrochemical oxidation of 33 with addition of sodium bicarbonate to the reaction medium gave a higher yield of product, e.g., the imine 34a (34%) in addition to a 30% yield of the dimerized product identified as 35. The structure of 35 is supported by its 1H NMR spectrum (500 MHz): δ 7.9 (d, J = 7.0 Hz, 2 H), 7.5 (t, J = 7.0 Hz, 1 H), 7.4 (t, J = 8.0
Hz, 2 H), 7.25 (d, J = 7.0 Hz, 2 H), 7.2 - 7.1 (m, 3 H), 6.9 (AB q, dv = 27 Hz, J = 8.0 Hz, 4 H), 6.6 (AB q, dv = 82 Hz, J = 10.0 Hz, 4 H), 2.2 (s, 3 H), 1.6 (s, 3 H). In addition, hydrolysis of the imine moiety with 5%AcOH/THF at room temperature gave the dienone 36 in 55% yield as a stable white crystalline solid. The $^{13}$C NMR (50 MHz) and combustion analysis of 36 confirmed the dimeric nature and elemental composition of 35: 185, 172, 151, 138, 138, 137, 131, 130, 128, 127, 127, 60, 27, 21; Anal. calcd for C$_{21}$H$_{19}$O$_2$N: C, 79.47; H, 6.03. Found: C, 79.48; H, 6.01. It is important to note the $^{13}$C peak at 60 ppm of 36 denotes the dimer as linked through the amide nitrogen, an observation consistent with the $^{13}$C NMR spectra of the structural derivative, 4-$N$-benzoylamino-4-methyl-2,5-cyclohexadienone and the ortho-dimerization.
Figure 2. 500 MHz $^1$H NMR Spectrum of 35
Figure 3. 50 MHz $^{13}$C NMR Spectrum of 35
Figure 4. 200 MHz $^1$H NMR Spectrum of 36
Figure 5. 50 MHz $^{13}$C NMR Spectrum of 36
product illustrated in Scheme X of the Introduction.

To further elucidate the experimental parameters of the above reaction, the anodic oxidation was performed at variable temperatures, concentration of starting amide, and current density. As shown in Table 4 below, manipulation of the oxidation conditions has a dramatic effect on the relative product yield of imine 34a and dimer 35. Entries 1 - 3 best illustrate the effect of temperature on the product ratio. As described earlier, the anodic oxidation performed at 25 °C gave the products 34a and 35 in 34% and 30% yields, respectively. However, lowering the reaction temperature increased the selectivity for imine formation; 0 °C, 34a (48 %), 35 (13 %); -15 °C, 34a (60 %), 35 (0%). Interestingly, increasing the concentration of the starting amide had a leveling effect on the product yields as shown in entries 4 - 6. In entry 4, a five-fold concentration increase (0.005 M to 0.025 M) of 33 at 0 °C decreased the yield of imine 34a from 48% to 31% and increased the formation of dimer 35 from 13% to 37% yield. However, a further increase in concentration of 33 up to 0.125 M (entries 5 and 6) contributed to no additional changes in the product yields.

With respect to current density (entries 7 and 8), an increase of current from 0.15 to 1.0 amperes had the beneficial effect of increasing the yield of imine 34a, e.g., 31% (entry 4) to 52% yield (entry 7), while dimer formation decreased. However, the leveling effect is still observed with an increase in concentration of starting amide 33 shown in entry 8. It should also be noted increased amounts
of electrolyte (10% LiClO$_4$) were necessary at higher currents to adequately control the temperature of the reaction mixture. In addition the effect of base (NaHCO$_3$) concentration and % volume of water in the methanolic solution were not investigated due to the detailed studies reported earlier. Therefore, the anodic oxidations

Table 4. Anodic Oxidations of $N$-Benzoyl-4-Aminotoluene

with NaHCO$_3$ in 5% H$_2$O:CH$_3$OH Solution

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temp (°C)</th>
<th>33 (M)</th>
<th>LiClO$_4$ (wt %)</th>
<th>I (mA)</th>
<th>% Yield$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-15.0</td>
<td>0.005</td>
<td>2.0</td>
<td>150</td>
<td>60.0</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
<td>0.005</td>
<td>2.0</td>
<td>150</td>
<td>48.0</td>
</tr>
<tr>
<td>3</td>
<td>25.0</td>
<td>0.005</td>
<td>2.0</td>
<td>150</td>
<td>34.0</td>
</tr>
<tr>
<td>4</td>
<td>0.0</td>
<td>0.025</td>
<td>2.0</td>
<td>150</td>
<td>31.0</td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
<td>0.050</td>
<td>2.0</td>
<td>150</td>
<td>28.0</td>
</tr>
<tr>
<td>6</td>
<td>0.0</td>
<td>0.125</td>
<td>2.0</td>
<td>150</td>
<td>33.0</td>
</tr>
<tr>
<td>7</td>
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<td>10.0</td>
<td>1000</td>
<td>52.0</td>
</tr>
<tr>
<td>8</td>
<td>0.0</td>
<td>0.050</td>
<td>10.0</td>
<td>1000</td>
<td>37.0</td>
</tr>
<tr>
<td>9</td>
<td>40.0</td>
<td>0.025</td>
<td>2.0</td>
<td>150</td>
<td>17.0</td>
</tr>
</tbody>
</table>

$^a$Oxidations were conducted with a 0.5 x 3 cm Pt cathode and a 5 cm diameter x 5 cm 45-mesh Pt anode. $^b$Reported yields were calculated by isolation via column chromatography.
were performed in the presence of a 10-fold excess of NaHCO$_3$ by weight relative to starting amide in 5% H$_2$O-CH$_3$OH solution.

In summary, the maximum yield of imine formation 34a is obtained by performing the anodic oxidation at lower temperatures (preferably at -15 °C) with a starting amide concentration of 5 x 10$^{-3}$ M using a constant current of 0.15 amperes. However, when higher concentrations of starting amide are desirable, a relative increase in current density is suggested for comparable yields of product, although variable reaction temperatures at higher current density becomes problematic. In this regard, incremental additions of the amide (1.0 mmol) per theoretical time may be a desirable alternative; the method utilized in the methanolic oxidation of N-benzoyl-p-methoxyaniline.$^{48}$

With most of the reaction conditions associated with the anodic oxidation of N-benzoyl-p-toluidine 33 in 5% H$_2$O-CH$_3$OH fully investigated, oxidations of other p-alkyl- and p-arylanilide derivatives were performed with the optimal reaction conditions described above. As shown in Table 5, the yields of the p-benzoquinol ether imines 37a - 45a ranged for the low end, a 17% yield for 37a up to an 80% yield for the imine 41a. Typically the lower yields were obtained with the N-acetyl-p-benzoquinol ether imines, e.g., 37a, 42a, and 44a, while the N-benzoyl-p-benzoquinol ether imine derivatives were generally isolated in much higher yield. Most importantly, the methyl ether analogue of 22, e.g., 44a, could be synthesized and isolated in 43% yield with upwards of 250 mg of material isolated in one run (see Experimental Section for details).
Table 5. Anodic Oxidation Products of Anilide Derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Structure 1" /></td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Structure 2" /></td>
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<td>3</td>
<td><img src="image" alt="Structure 3" /></td>
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<td>4</td>
<td><img src="image" alt="Structure 4" /></td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Structure 5" /></td>
<td>80</td>
</tr>
</tbody>
</table>

41a: R=Ph  
42a: R=CH<sub>3</sub>
Table 5. (continued)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td><img src="" alt="Image of compound 6" /></td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>43a: R=Ph</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>44a: R=CH₃</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><img src="" alt="Image of compound 7" /></td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>45a</td>
<td></td>
</tr>
</tbody>
</table>

a The anodic oxidations were conducted with a constant current of 0.15 A at -15 °C using 5.0 mM concentration of starting amide in 5% H₂O-CH₃OH solution with NaHCO₃.
b A 10% yield of 4-methoxy-4-phenyl-2,5-cyclohexadienone was also isolated during purification of 42a and a 12% yield of the respective dienone for 44a (see Experimental Section for details).

However the stability of compounds 37a - 45a mandated a short storage lifetime. For example, considerable decomposition of 44a was observed after one week of storage at -20 °C under nitrogen. In addition, the N-acetyl-p-benzoquinol ether imines are highly susceptible to imine hydrolysis when left open to atmosphere while the
N-benzoyl-p-benzoquinol ether imine derivatives are much less reactive to hydrolysis.

The greater reactivity for imine hydrolysis in the N-acetyl-p-benzoquinol ether imine series of compounds initially translated into isolation difficulties when using adsorption chromatography. For example, attempts to isolate and purify 37a with silica gel and neutral alumina column chromatography gave appreciable amounts of the respective dienone, e.g., 4-methyl-4-methoxy-2,5-cyclohexadienone. However, performance of adsorption chromatography with the less activated Florisil support greatly decreased the propensity for imine hydrolysis although small amounts of the respective dienones of compounds 42a and 44a were obtained during purification (see ref. b in Table 5).

The successful synthesis of the N-acylated-p-benzoquinol ether imines described above, prompted further investigation into the anodic oxidation of the parent amide systems in aqueous solvents in an attempt to synthesize the p-benzoquinol imine analogues. Initial studies were again done with N-benzoyl-p-toluidine 33 utilizing a number of non-nucleophilic solvents (CH\textsubscript{3}CN, DMF, THF) with variable ratios of water. As illustrated above in Table 6, an appreciable effect on product yield from the anodic oxidation of 33 was not observed (entries 1-3) with increased water content in CH\textsubscript{3}CN. However, the electrochemical oxidation in 30% H\textsubscript{2}O:THF and 30% H\textsubscript{2}O:DMF solvent systems (entries 7,8) gave reduced yields of the imine 34b as well as
Table 6. Anodic Oxidations of N-Benzoyl-p-Toluidine with NaHCO₃ in H₂O-CH₃CN, DMF, and THF Solutions.

![Structure](image)

<table>
<thead>
<tr>
<th>Entry, b</th>
<th>Temp. (°C)</th>
<th>H₂O:Solvent c (M)</th>
<th>33</th>
<th>34b</th>
<th>% Yield d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.0</td>
<td>10.0 : CH₃CN 0.005</td>
<td>19.0</td>
<td>35.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25.0</td>
<td>30.0 : CH₃CN 0.005</td>
<td>6.0</td>
<td>44.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25.0</td>
<td>50.0 : CH₃CN 0.005</td>
<td>12.0</td>
<td>37.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10.0</td>
<td>30.0 : CH₃CN 0.005</td>
<td>10.0</td>
<td>37.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25.0</td>
<td>30.0 : CH₃CN 0.025</td>
<td>22.0</td>
<td>31.0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>25.0</td>
<td>30.0 : CH₃CN 0.025</td>
<td>3.0</td>
<td>36.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>25.0</td>
<td>30.0 : THF 0.005</td>
<td>9.0</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>25.0</td>
<td>30.0 : DMF 0.005</td>
<td>16.0</td>
<td>22.0</td>
<td></td>
</tr>
</tbody>
</table>

a A 50% yield of recovered amide 33 after 2 x theoretical time was obtained with the oxidation conditions in entry 7 and a 25% recovered yield of 33 in entry 8. b Entries 1 - 5, 7 and 8 were conducted at a constant current of 0.15 A whereas entry 6 was performed at a constant current of 1.0 A (see Experimental Section for details). c Solutions were prepared by % volume H₂O : CH₃CN, THF or DMF. d Reported yields were determined by isolation via column chromatography.
the dimer 35. Interestingly, the current efficiencies for entries 7 and 8 (see ref a. in Table 6) were much lower (20-25% c.e.) relative to the anodic oxidation performed in water-acetonitrile (70-75% c.e.). In the case of entry 8, this phenomenon may be the result of solvent oxidation since DMF has an oxidation potential (1.2 vs S.C.E.) comparable to the amide 33 (see Table 8 in Discussion). However with THF, direct anodic oxidation of the solvent is unlikely and hydrogen abstraction of THF via methoxy radical is probably the more important side-reaction.

As described earlier on the methanolic oxidations of 33, the product ratios of p-benzoquinol imine 34b to dimer 35 were dependent upon reaction temperature, concentration of starting amide 33, and current density. However, as shown in Table 6, a temperature effect was not observed with the anodic oxidation of 33 in 30% H₂O:CH₃CN solution (entries 2 and 4) in addition to a negligible concentration effect shown in entries 2 and 6. With respect to current density, an increase in the current from 0.15 to 1.0 amperes (entry 6), dramatically decreased the yield of dimer 35, however no appreciable effect was observed on the formation of imine 34b. In brief summary, the anodic oxidation of N-acylated anilides is best performed in 30% H₂O:CH₃CN solution (unless solubility of the starting amide dictates lower water content) with a constant current of 0.15 A at room temperature using 2% LiClO₄ as electrolyte and NaHCO₃ as base.

To further elucidate the utility of the above electrochemical reaction and ultimately synthesize the benzoquinol imine 22, the
anilides used in the previous study were employed. As illustrated in Table 7, the constant current electrochemical oxidations in 30% H$_2$O:CH$_3$CN solution gave the N-benzoyl-p-benzoquinol imines in 30 - 45% isolated yields, 20 - 30% lower yields relative to the methyl ether analogs. In addition attempts to anodically oxidize the N-acetyl-anilides under these conditions resulted in a complex mixture of side-products or extremely low yields of the desired product. For example the anodic oxidation of N-acetyl-5-aminooindane 46 in 30%

Table 7. Anodic Oxidation of Anilides in 30% H$_2$O:CH$_3$CN Solution.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Yield (%)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Image" /></td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Image" /></td>
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</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Image" /></td>
<td>45</td>
</tr>
</tbody>
</table>
Table 7. (continued).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td><img src="image1.png" alt="Image" /></td>
<td>30&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td><img src="image2.png" alt="Image" /></td>
<td>32</td>
</tr>
</tbody>
</table>

<sup>a</sup>The above compounds are approximately 95% pure by <sup>1</sup>H NMR due to small amounts of dienone side-product inseparable by column chromatography. <sup>b</sup>4-<i>N</i>-benzoylamino-6-phenylphenol was also obtained in 15% yield. <sup>c</sup>The anodic oxidation was performed in 1:1:6 H<sub>2</sub>O:DMF:CH<sub>3</sub>CN solution (see Experimental Section for details).

H<sub>2</sub>O:CH<sub>3</sub>CN with NaHCO<sub>3</sub> at room temperature gave the p-benzoquinol imine 46b in 4% yield in addition to a 4% yield of the dienone 46c (Scheme XV). Interestingly the carbamate aromatic amines give respectable yields of the p-benzoquinol imine such as 38b (entry 1).

In addition to lower isolated yields for the oxidation of <i>N</i>-acylated anilides in aqueous media, the p-benzoquinol imines
exhibited a dramatic increase in the proclivity for a dienone-phenol rearrangement relative to their methyl ether counterparts. For

**Scheme XV. Anodic Oxidation of N-Acetyl-5-Aminoindane.**

![Scheme image]

example anodic oxidation of N-benzoyl-4-sec-butyl-aniline 40 afforded a 45% yield of the rearrangement product, e.g., 4-N-benzoylelamino-6-sec-butylphenol 40c, while oxidation of N-benzoyl-

**Scheme XVI.**

![Scheme image]

4-aminobiphenyl 41 gave 41b and 41c in 30% and 15% yields respectively. Authentic 40c and 41c were prepared as outlined above and the melting point and spectroscopic properties consistent with the structural
assignment (Scheme XVI). Thus p-benzoquinol imines with p-substituents with good migrating ability such as aryl or tertiary alkyl groups are highly susceptible to the dienone-phenol rearrangement.

Although partial loss of material is due to the aryl migration described above, the lower oxidation yield of 41b relative to 41a warranted further analysis. The anodic oxidation of 41 in aqueous media was monitored by high pressure liquid chromatography, to in effect, determine the current efficiency of the reaction, and the extent of decomposition of 41b during the electrochemical oxidation, isolation and reaction work-up. The most apparent observation upon analysis of the bar graph illustrated in Figure 2, is the efficient oxidation of starting amide, e.g., a 92% consumption of 41 at 1.0 theoretical time. However it appears complete oxidation of the amide 41 will dictate partial consumption of the product 41b during the electrochemical reaction. Thus the instability of the p-benzoquinol imine 41b to the reaction conditions may be attributed to the lower isolated yield, although a comparative analysis was not attempted on the anodic oxidation of 41 in methanolic solvent. In addition the HPLC analysis gave an analytical yield of 55% for 41b at 1.3 theoretical time, indicating negligible decomposition during the isolation and reaction work-up.

Although 41b could be isolated in moderate yield, albeit with some difficulty due to the acid sensitive nature of the compound, attempts to synthesize and isolate the p-benzoquinol imine 43b proved to be very difficult. For example the anodic oxidation of 43
Theoretical Time

Figure 2. HPLC Analysis\textsuperscript{a, b} of the Anodic Oxidation of 41 in 1:1:6 H\textsubscript{2}O:DMF:CH\textsubscript{3}CN Solution

\textsuperscript{a}The bold-faced bars denote consumption of the amide 41, shaded bars denote formation of the product 41b. \textsuperscript{b}2-chloronapthalene was used as internal standard.\textsuperscript{51}

in a single cell apparatus with 10\% H\textsubscript{2}O:CH\textsubscript{3}CN at room temperature gave a complex mixture of products. Additional efforts for improvement by lowering the reaction temperature, increasing the water content or adding DMF to the reaction solvent to increase solubility also proved unsuccessful. The next step was to investigate the electrochemical oxidation of 43 in a divided cell apparatus. Preliminary investigations again gave complex product mixtures with variable
ratios of H₂O:CH₃CN. However when the anodic oxidation of 43 was performed in a divided cell using a 1:1:8 ratio of H₂O:DMF:CH₃CN solvent system in the presence of a 20-fold excess of NaHCO₃ at 0 °C, a product characterized as p-benzoquinol imine 43b was isolated in 20% yield (Scheme XVII, Figure 3). The instability of the product, unfortunately, made isolation and handling of 43b very difficult, and subsequently restricted its complete characterization. However analysis vis a' vis, 500 MHz ¹H and 125 MHz ¹³C NMR, provided strong evidence for the structure: δ 7.9 (d, J = 8.0 Hz, 2H), 7.6 – 7.4 (m, 4 H), 7.3- 7.2 (m, 4 H), 6.4 (dd, J = 10.0, 1.7 Hz, 1 H), 6.2 (d, J = 1.6 Hz, 1H), 4.1 (dd, J = 16.0, 2.0 Hz, 1 H), 3.6 (d, J = 18.0 Hz, 1 H); 181, 158, 142, 142, 134, 131, 131, 130, 129, 128, 126, 124, 118, 73, 36.
Figure 7. 500 MHz $^1$H NMR Spectrum of 43b in d$_6$-acetone
Figure 8. 500 MHz $^{13}$C NMR Spectrum of 43b in $d_6$-acetone

- a: carbonyl carbon of amide 43
- b: methylene carbon of amide 43
In summary, painstaking efforts to isolate and analyze the 43b resulted in large amounts of frustration and futility. For example, the compound exhibited an extreme sensitivity to very mild acidic conditions, and readily decomposed with adsorption chromatography. In addition, the reactivity of 43b dictated that all glassware be thoroughly washed with base (Et$_3$N or NH$_2$OH) prior to contact, apparently to neutralize the acidic surface of the glassware. Also attempts to purify the compound via recrystallization proved
unsuccessful as well as the characterization of the numerous decomposition products. Therefore, due to the highly reactive nature of the compound, additional efforts to satisfactorily characterize 43b were not continued.
Mechanistic Discussion

The results presented herein demonstrate that the electrochemical oxidation of p-alkylanilides with NaHCO₃ in methanolic and aqueous solvents will form the respective p-benzoquinol and quinol ether imines in moderate to good yield. In addition, this investigation examined certain experimental conditions to further elucidate the mechanism of the anodic oxidation reaction. As discussed earlier in the introduction, previous investigations introduced the EEC_rC_p mechanism (the preferred pathway for the anodic oxidation of 1,4-dimethoxybenzene derivatives in KOH-methanol solution) as a preliminary rationalization for the anodic oxidation of p-methoxybenzanilide derivatives. In this mechanistic sequence, initial oxidation of the aromatic ring generates a radical cation intermediate, followed by para-coupling with methoxy radical at the electrode surface to form a cationic intermediate (see Scheme XII in Introduction). This intermediate may then undergo 1,2-addition of methanol to form the 1,4-substituted adduct or, in the case of the anodic oxidation of p-methoxyanilides, undergo proton removal to give the p-benzoquinone imine ketal.

However, if the anodic oxidation of p-substituted anilides were viewed in a manner similar to the electrochemical oxidation of phenolic derivatives, additional mechanistic scenarios may be described (Scheme XVIII). For example it is commonly regarded that the oxidation of phenols 1a begin with the formation of the radical cation intermediate IIa followed by proton loss to give the phenoxy
Depending on the reaction conditions, the phenoxy radical may then undergo further oxidation to form the phenoxonium ion \( \text{IVa} \) or, very commonly, undergo ortho-substituted dimerization. This scenario may also be utilized for the rationalization of the electrochemical oxidation of \( p \)-substituted anilides.

Scheme XVIII. Electrochemical Oxidation of Phenols and Benzanilides.

As noted previously, the product formation of the anodic oxidation of \( N \)-benzoyl-4-aminotoluene \( 33 \) in methanolic solution exhibits a dramatic temperature dependence, e.g., at \(-15^\circ\text{C}\), \( 34a \) is formed in 60% yield whereas at \( 40^\circ\text{C} \), \( 35 \) is obtained in 55% yield in addition to a 17% yield of \( 34a \) (Table 4, p 33). To determine whether formation of \( 35 \) at higher temperature was in effect the result of the dimerization of an anilide radical \( \text{Ila} \) or due to ionization of \( 34a \) followed by capture of the parent amide, the imine \( 34a \) and the amide \( 33 \)
Table 8. Oxidation Potentials of Benzamide Derivatives

![Structural Diagram](https://via.placeholder.com/150)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compd</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$E_{p/2}$ (V)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
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<td>30a</td>
<td>Ph</td>
<td>OCH$_3$</td>
<td>1.25</td>
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<tr>
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<td>CH$_3$</td>
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<td>CH$_3$</td>
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<td>5</td>
<td>39</td>
<td>Ph</td>
<td>C$_2$H$_5$</td>
<td>1.48</td>
</tr>
<tr>
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<td>40</td>
<td>CH$_3$</td>
<td>s-C$_4$H$_9$</td>
<td>1.43</td>
</tr>
<tr>
<td>7</td>
<td>41</td>
<td>Ph</td>
<td>Ph</td>
<td>1.40</td>
</tr>
<tr>
<td>8</td>
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<td>Ph</td>
<td>b</td>
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<tr>
<td>9</td>
<td>45</td>
<td>Ph</td>
<td>c</td>
<td>1.38</td>
</tr>
</tbody>
</table>

<sup>a</sup> Measurements were taken with approximately 3.5 mM solution of amide in CH$_3$CN vs Ag/AgCl reference electrode. <sup>b</sup>2-aminofluorene. <sup>c</sup>5-aminoindane.
were dissolved in 5% H$_2$O:CH$_3$OH solution, lithium perchlorate and NaHCO$_3$ added, and the mixture heated to 40 °C. Analysis of the product mixture, however, did not detect formation of the dimer 35 after 3.0 h, and thus excluding the latter hypothesis.

Furthermore, cyclic voltammetry experiments were undertaken to determine the oxidation potentials of the parent amides relative to methanol (E$_{1/2}$ = 2.50 V)$^{53}$. As shown in Table 8 the oxidation potentials of the p-alkyl- and p-arylanilides are much lower (E$_{p/2}$ = 1.4 to 1.5 V) relative to methanol, and interestingly the fluorene nucleus is even lower (E$_{p/2}$ = 1.20), undoubtedly due to the additional benefit of conjugation with the tethered aromatic ring. Thus the large difference in oxidation potentials suggests the oxidation of methanol may not generate methoxy radical in sufficient concentrations to effectively compete with dimerization.

In addition, performing the anodic oxidation of 33 at higher current densities substantially increased the yield of methyl ether imine 34a. This observation may be attributed to the following two hypotheses: (a) higher current density increases the concentration of methoxy radical, which in turn kinetically favors formation of 34a relative to 35, or (b) at higher current density, oxidation of the dimer followed by methanol capture of the cationic intermediate IVb subsequently increases the yield of 34a. To examine the latter hypothesis, the dimer 35 was anodically oxidized using a constant current of 1.0 A at 0 °C with NaHCO$_3$ as base and the reaction analyzed by HPLC. However the electrochemical oxidation resulted in complete
decomposition of the dimer 35 and no observable formation of the imine 34a. In addition, the anodic oxidation of 33 in methanolic solution was examined using constant potential conditions. Interestingly, when performed at a constant potential of 2.75 V, a 52% yield of the p-benzoquinol ether imine 34a was obtained in addition to a 30% yield of recovered amide 33 and a complex mixture of side products; however at 1.75 V, the imine 34a was isolated in 33% yield, along with the dimer 35 (15%) and recovered amide 33 (20%).

Therefore the experimental evidence suggests that at lower current densities, the higher oxidation potential of methanol results in a lower net concentration of methoxy radical relative to oxidized amide IIIb and therefore favors dimerization. However, manipulation of the reaction conditions can favor the formation of the p-benzoquinol ether or quinol imine by: (1) lowering the reaction temperature which favors p-benzoquinol imine formation by decreasing the rate of dimerization and/or (2) perform the anodic oxidation at higher current density, which increases methoxy radical concentration and subsequently increases the rate of p-benzoquinol imine formation.

In addition to the above experimental data, a mechanistic scenario for the anodic oxidation of p-substituted benzamides must incorporate a rationalization for the lower isolated yields of the \( N \)-acetyl-p-benzoquinol ether and quinol imines in both aqueous and methanolic solutions. For example, the anodic oxidation of \( N \)-benzoyl-4-aminotoluene 33 and \( N \)-acetyl-4-aminotoluene 37 in methanol gave the p-benzoquinol ether imines 34a and 37a in 60% and 17% yields.
respectively (Table 5, p 35); and as illustrated earlier, electrochemical oxidation of 45 in aqueous acetonitrile gave the p-benzoquinol imine 45b in 4\% yield in addition to a 4\% yield of the respective dienone 46c (Scheme XV, p 42).

A suitable mechanism for the anodic oxidation of p-alkyl- and p-arylbenzanilides is illustrated in Scheme XIX. The sequence below begins with initial one electron oxidation of the parent amide (I) which generates the radical cations (II) or (III), both strong acids. In mildly basic media, this intermediate may then undergo deprotonation to give the aryl radicals (IV) (when R\textsuperscript{1} = Ph) or (VI). With the intermediate (V), two different reaction paths may then follow, dimerization or coupling with methoxy radical to form the p-benzoquinol ether or quinol imine. However when performing the electrochemical oxidation on N-acetylanilides (R\textsuperscript{1} = CH\textsubscript{3}), an alternative sequence can become competitive with deprotonation, e.g., deacylation of the radical cation (III) via addition of solvent to the amide carbonyl and release of methyl acetate to generate the aniline radical (V). The intermediate (V) may then undergo further oxidation or dimerization. Evidently the conjugating ability of the acyl linkage, e.g., the benzoyl and carbamate moieties, sufficiently deactivate the amide carbonyl of the radical cation (III) to nucleophilic attack by the solvent and subsequently inhibit deacylation. Finally the solvent may add to the ipso carbon of the amide nitrogen forming the intermediate (VII) which can then couple with methoxy radical to give (VIII). Depending on the solvent (R\textsuperscript{2} =}
Scheme XIX. A Proposed Mechanism for the Anodic Oxidation of Benzanilides in NaHCO₃.
H), intermediate (VIII) may then decompose to the dienone (XI). This explains the dienone side-products observed with the anodic oxidations in aqueous acetonitrile.

Furthermore the acidities of the methyl and amide protons of radical cations (II) and (III) in NaHCO₃ apparently dictate which resonance form predominantly contributes to the chemistry. This became more evident with the anodic oxidation of 47 in 5% H₂O:MeOH solution (Scheme XX). Under constant current conditions, the oxidation of 47 gave the mixed ketal imine 48 in 48% yield, in addition to the p-benzoquinone imine ethylene glycol ketal 49 in 17% yield. The product ratio of this reaction can be rationalized according to the mechanism discussed above in Scheme XIX. Formation of 48 is analogous to the (I) - (III) - (V) transformation followed by methoxy radical coupling to form the resultant product. The p-benzoquinone imine ketal 49, however, may be the result of the capture of radical
cation 47a (analogous to intermediate II in Scheme XXI) with the ethylene glycol tether to form the ketal radical 47b (Scheme XIII). Coupling with methoxy radical to form 47c and elimination of methanol with then give 49. From this experiment, a rough extrapolation would suggest the radical cation (II) would account for 20% of the chemistry in the anodic oxidation of p-alkylanilides, an approximation close to

Scheme XXI.

the difference in isolated yields for the p-benzoquinone imine ketal 32a (92%) and 4-methyl-4-methoxy-p-benzoquinone imine 34a (60%).
Finally, the difficulties associated with the synthesis of 43b warrant a brief mechanistic discussion. As was mentioned earlier, the successful synthesis of 43b dictated that the anodic oxidation be performed in a divided cell apparatus with a 1:1:8 solution of H₂O:DMF:CH₃CN, with DMF being an important reagent (see Figure 3). Interestingly, the oxidation potential of DMF (E₁/₂ = 1.20 V vs S.C.E.) and N-benzoyl-2-aminofluorene 43 (Eₚ/₂ = 1.20 V vs Ag/AgCl) are comparable. Since the oxidation potential of water is much higher, the similarity in oxidation potentials of starting amide and DMF may in effect dictate the outcome of the reaction. In addition, previous studies on the cyclic voltammetry of the electrochemical

Scheme XXII.
oxidation of DMF in methanol, reported an irreversible one-electron oxidation of the amide as the rate determining step in the synthesis of N-methoxy-N-methylformamide. Therefore the mechanism illustrated in Scheme XXII is consistent with experimental and literature data. However the rationalization shown above should only be viewed as tentative since mechanistic investigations were not fully pursued.

Interestingly a model study of the anodic oxidation of 33 in a 1:1:8 solution of DMF:H₂O:CH₃CN did in fact change the product composition, although not dramatically. For example, oxidation of a 1.0 mM solution of 33 in a 2% lithium perchlorate solution with NaHCO₃ gave a 34% yield of the p-benzoquinol imine 34b in addition to a 30% yield of the dimer 35 at room temperature. Recall the oxidation in 30% H₂O:CH₃CN gave a 44% and 6% yield of 34b and 35, respectively. In addition varying the reaction temperature had no effect on the product yield.

In summary, the constant current oxidation of p-alkyl- and p-arylbenzanilides in methanolic solution with NaHCO₃ gave moderate to excellent yields (45% - 80%) of the respective 4-substituted p-benzoquinol methyl ether imines. However the anodic oxidation in aqueous acetonitrile gave much lower yields (30% - 45%) of 4-substituted p-benzoquinol imines. In addition the p-benzoquinol imines displayed a marked increase in reactivity and therefore the compounds were difficult to isolate. Furthermore attempts to synthesize the biological metabolite 22 were not fully realized. However the successful synthesis of relatively large quantities of the
methyl ether analogue 44a did provide enough material for biologically related investigations.
Experimental

General Procedures

Melting points were determined in capillaries in a Thomas-Hoover "Unimelt" apparatus and are uncorrected. IR spectra were determined on a Perkin-Elmer Model 283B spectrometer. Unless noted otherwise, the $^1$H NMR spectra were measured on a Bruker 200 MHz $^1$H NMR using deuteriochloroform as solvent and residual chloroform as standard. Mass spectral and exact mass measurements were obtained by Mr. Richard Weisenberger on a Kratos MS-30 spectrometer connected to a DS-55 data system. Combustion analyses were performed by Scandinavian Microanalytical Laboratory, Herlev, Denmark, and M-H-W Laboratories, Phoenix, Arizona. All reagents or compounds not explicitly referenced were obtained from the Aldrich Chemical Co. Alumina and silica gel (Kieselgel 60 230-400 mesh) were obtained from E. Merck Co and Florisil (100 - 200 mesh) purchased from Fischer Scientific Co. Thin-layer chromatography (TLC) was done using Merck silica gel 60 F$_{254}$ pre-coated aluminum backed plates, 0.2-mm thickness. Visualization was by UV or by spraying with 5% ethanolic phosphomolybdic acid and then heating. Tetrahydrofuran was purified by distillation from benzophenone ketyl. Throughout the Experimental Section the following abbreviations are used: petroleum ether, bp 35-60 °C (PE), hexanes, bp 68-69 °C (H), ethyl acetate (EtOAc), tetrahydrofuran (THF), p-toluene sulfonic acid (p-TsOH), and thin layer chromatography (TLC). Extractive workup refers to extraction of the material into the
indicated solvent, washing the organic layer with brine solution, drying over Drierite (CaSO₄), concentration in vacuo, and drying to constant weight under vacuum (1-2 Torr). Unless noted otherwise all preparative anodic oxidations were performed under constant current conditions in a single-cell apparatus in reagent grade methanol, acetonitrile and water, using a cylindrical platinum anode (5 cm x 3.5 mm diameter, 50 mesh screen), a rectangular platinum sheet cathode (8 x 8 mm) and a Kepco JQE 36-3 M power supply. Divided cell oxidations were performed in an H-type divided cell apparatus using a cylindrical platinum anode (5 cm x 2.5 cm diameter) and a platinum wire cathode. Controlled temperature oxidations were performed using a water-methanol jacketed beaker equipped to a Brinkman/MGW Lauda RC-3 temperature bath.

The starting amides, 8, 33, 37-46 were prepared either by simple acylation of the aniline or reduction of the nitro-compound via catalytic hydrogenation using 10% Pt/C catalyst followed by acylation. Sample experimentals are provided on the following page.

**Preparation of 8**

2-Nitrofluorene (7 g, 0.033 mol) was dissolved in THF (75 mL) and placed in a Paar hydrogenation bottle. A 10% Pt/C catalyst (350 mg) was added and the
contents placed under hydrogen. The mixture was hydrogenated for 8.0 h and monitored by TLC (40% EtOAc/H). Upon completion the mixture was filtered through celite and the filtrate added to a solution of Et₃N (5 mL, 0.045 mol) and THF (50 mL). Acetic Anhydride (4 mL, 0.045 mol) was added and the solution heated to boiling on a steam bath for 0.5 h with intermittent swirling. The solvent was then removed in vacuo giving a yellow brown solid. Trituration with Et₂O (50 mL) gave a light yellow solid (7.2 g, 99% yield) as product, mp 188.0 - 189.0 °C (lit mp 56 192.0 - 194.0 °C).

Preparation of 33

4-Nitrotoluene (15 g, 0.11 mol) was dissolved in THF (100 mL) and placed in a Paar hydrogenation bottle. A 10% Pt/C catalyst was added and the contents placed under hydrogen (65 psi). The reaction was monitored by TLC (40% EtOAc/H) and complete after 10 h. The reaction mixture was filtered through celite and the filtrate added to a solution of Et₃N (11 ml, 0.1 mol) and THF (50 mL). Benzoyl chloride (9 mL, 0.1 mol) was then added slowly, forming a yellow precipitate and the mixture heated on a steam bath to boiling for 0.5 h. Upon cooling, H₂O (400 mL) was added forming a precipitate, filtered and dried, giving a white solid (21.5 g, 93% yield) as product, mp 154.0 - 155.0 °C (lit mp 57 157.0-158.0 °C).
Anodic Oxidation Studies (Table 3)

Preparation of 34a

Entry 1. 4-(N-Benzoylamino)toluene

(215 mg, 1.0 mmol, Rf = 0.60, 20% EtOAc/H)
was placed in a 300 mL jacketed beaker, 
dissolved in 5% H₂O:CH₃OH solution (200 
ml) and cooled to -15 °C. Lithium perchlorate trihydrate (4.0 g) and freshly 
ground NaHCO₃ (6.0 g) were added and the mixture anodically oxidized at a constant current of 0.15 A (9 - 10 V). The reaction was monitored by TLC (2% EtOAc/CH₂Cl₂ used as eluant) and complete after 35 minutes. The reaction mixture was then filtered through a plug of glass wool, H₂O (100 mL) added, and extractive workup with CH₂Cl₂ 
(3 x 50 mL) gave the crude product as a yellow oil (230 mg). Purification was then performed by column chromatography with a 
Florisil support (10 cm x 1 cm column) and 15% EtOAc/H as eluant. The product 34a (Rf = 0.54, 2% EtOAc/CH₂Cl₂) was isolated as a light yellow oil (144 mg, 60% yield) with absolute purity and spectral properties identical to authentic sample.⁵⁸ IR (KBr) 1653, 1599, 1246, 1092, 1060, 716 cm⁻¹; ¹H NMR δ 7.9 (d, J = 8.0 Hz, 2 H), 7.5 - 7.3 (m, 3 H), 6.4 (s, 4 H), 3.1 (s, 3 H), 1.4 (s, 3 H); ¹³C NMR δ 181, 157, 148, 134, 134, 131, 130, 128, 73, 54, 28.
Entry 7. 4-(N-Benzoylamino)toluene (1.06 g, 5.0 mmol) was placed in a 300 mL jacketed beaker, 5% H₂O:CH₃OH (200 mL) added and the solution stirred vigorously until completely homogeneous. The solution was then cooled to -3 °C (temp. bath -10 °C) and lithium perchlorate trihydrate (20.0 g) and freshly ground NaHCO₃ (10.0 g) added. The mixture was electrolyzed at a constant current of 1.0 A (9 - 10 V) warming the solution to 0 °C. After 30 minutes the reaction was stopped, followed by standard workup giving a dark yellow oil (1.2 g). Purification was then performed with column chromatography using a Florisil support (15 cm x 2 cm column) and 15% EtOAc/H as eluant. Compound 34a was the first component eluted giving a light yellow oil (623 mg, 52% yield) as product, followed by isolation of the dimer 35 as a yellow oil (90 mg, 9% yield).

Preparation of 35

Entry 8. N-Benzoyl-4-aminotoluene (1.06 g, 5.0 mmol) was placed in a 300 mL jacketed beaker, dissolved in a 5% H₂O:CH₃OH solution (200 mL) and warmed to 40 °C. Lithium perchlorate trihydrate (4.0 g) and freshly ground NaHCO₃ (6.0 g) were added and the mixture anodically oxidized at a constant current of 0.15 A (4 - 5 V). The reaction was monitored by TLC (2% EtOAc/CH₂Cl₂) and complete in 2.5 h. The reaction mixture was then filtered through a plug of glass wool, H₂O (200 mL) added and
extractive workup performed with CH$_2$Cl$_2$ (3 x 100 mL) to give a yellow oil (1.2 g). Purification was performed with column chromatography using a Florisil support (15 cm x 2 cm column) and 25% EtOAc/H as eluant. The first compound off the column was the imine 34a isolated as a light yellow oil (200 mg, 17% yield), followed by isolation of the dimer 35 (R$_f$ = 0.45, 40% EtOAc/H) as a yellow oil (562 mg, 53% yield) with 95% purity. IR (neat) 1653, 1598, 1580, 1510, 1341, 1247 cm$^{-1}$; $^1$H NMR (500 MHz) $\delta$ 7.9 (d, J = 7.0 Hz, 2 H), 7.5 (t, J = 7.0 Hz, 1 H), 7.4 (t, J = 8.0 Hz, 2 H), 7.25 (d, J = 7.0 Hz, 2 H), 7.2 - 7.1 (m, 3 H), 6.9 (AB q, $\Delta v$ = 27 Hz, J = 8.0 Hz, 4 H), 6.6 (AB q, $\Delta v$ = 82 Hz, J = 10.0 Hz, 4 H), 2.2 (s, 3 H), 1.6 (s, 3 H); $^{13}$C NMR $\delta$ 181, 172, 156, 152, 147, 138, 138, 133, 133, 131, 129, 128, 128, 128, 127, 123, 60, 28, 21; HRMS Calcd for C$_{28}$H$_{24}$O$_2$N$_2$: m/e 420.1832, obsd 420.1836.

Preparation of 36

The dimer 35 (125 mg, 0.3 mmol), acetone (5 mL), H$_2$O (1 mL) and CH$_3$CO$_2$H (1 mL) were placed in a 25 mL round-bottom flask and stirred at room temperature. The reaction was monitored by TLC (40% EtOAc/H used as eluant) and complete after 10 h. Saturated NaHCO$_3$ (5 mL) was then added and extractive workup performed with CH$_2$Cl$_2$ (2 x 10 mL). A brown oily solid (125 mg) was obtained which was purified by column chromatography with a silica
gel column (10 cm x 1 cm, 230 - 400 mesh) and 20% EtOAc/H as eluant. The product 36 (Rf = 0.39, 40% EtOAc/H) was isolated off the column as a pale white solid (48 mg, 53% yield), 150.0 - 152.0 °C. Two recrystallizations from Et₂O/H gave analytically pure material, mp 156.0 - 157.0 °C. IR (KBr) 1661, 1643, 1623, 1349, 858 cm⁻¹; ¹H NMR δ 7.3 - 7.2 (m, 7 H), 7.1 - 6.9 (AB q, dv = 11 Hz, 4 H), 6.2 (d, J = 10 Hz, 2 H), 2.2 (s, 3 H), 1.6 (s, 3 H); ¹³C NMR δ 185, 172, 151, 138, 138, 137, 131, 130, 128, 127, 127, 60, 27, 21; Anal. calcd for C₂₁H₁₉O₂N: C, 79.47; H, 6.03. Found: C, 79.48; H, 6.01.

Preparation of 37a

_N-Acetyl-4-aminotoluene_ (150 mg, 1.0 mmol) was dissolved in 5% H₂O:CH₃OH (200 mL) and the solution cooled to -15 °C. Lithium perchlorate trihydrate (4.0 g) and freshly ground NaHCO₃ (6.0 g) were then added and the mixture electrolyzed at a constant current of 0.15 A (4 - 5 V). The reaction was monitored by TLC (40% EtOAc/H) and complete after 35 minutes. Extractive workup was then performed with CH₂Cl₂ (2 x 75 mL) giving a yellow oil (160 mg) as crude product. The oil was impregnated on Florisil (1.2 g) and column chromatography performed with a Florisil support (10 cm x 2 cm column, 25% EtOAc/H used as eluant) and Et₃N (5 mL) flushed through the column prior to addition of material. The product 37a (Rf = 0.41, 40% EtOAc/H) was isolated as a light yellow
oil (31 mg, 17% yield) and spectral properties consistent with authentic sample.\(^{59}\) \(^1\)H NMR \(\delta 6.4\) (AB q, \(\Delta\nu = 23\) Hz, \(J = 10\) Hz, 4 H), 3.1 (s, 3 H), 2.2 (s, 3 H), 1.3 (s, 3 H).

**Preparation of 38a**

\(N\)-Methoxycarbonyl-4-aminotoluene

(165 mg, 1.0 mmol, \(R_f = 0.83, 4\%\) Et\(\text{OAc}/\text{CH}_2\text{Cl}_2\)) was placed in a 300 mL jacketed beaker, dissolved in 5% \(\text{H}_2\text{O}/\text{CH}_3\text{OH}\) solution (200 mL) and cooled to -15 °C. Lithium perchlorate trihydrate (4.0 g) and freshly ground NaHCO\(_3\) (6.0 g) were added and the mixture anodically oxidized at a constant current of 0.15 A (8 - 9 V). The reaction was monitored by TLC (4% Et\(\text{OAc}/\text{CH}_2\text{Cl}_2\) used as eluant) and complete after 35 minutes. The reaction mixture was then filtered through a plug of glass wool, \(\text{H}_2\text{O}\) (200 mL) added, and extractive workup with \(\text{CH}_2\text{Cl}_2\) (3 x 50 mL) gave the crude product as a yellow oil (175 mg). Purification was performed by column chromatography using a Florisil support (8 cm x 2 cm column) and 10% Et\(\text{OAc}/\text{H}\) as eluant. The product 38a (\(R_f = 0.61, 4\%\) Et\(\text{OAc}/\text{CH}_2\text{Cl}_2\)) was isolated off the column as a white solid (90 mg, 46% yield); mp 70.0 - 71.5 °C. IR (KBr) 1720, 1650, 1590, 1230, 1110, 1080, 840 cm\(^{-1}\); \(^1\)H NMR \(\delta 6.4\) (AB q, \(\Delta\nu = 16\) Hz, \(J = 10\) Hz, 4 H), 3.8 (s, 3 H), 3.1 (s, 3 H), 1.4 (s, 3 H); \(^13\)C NMR \(\delta 163, 158, 148,\) 72, 53, 53, 26; HRMS Calcd for \(\text{C}_{10}\text{H}_{13}\text{O}_3\text{N}: m/e 195.0892,\) obsd 195.0941.
Preparation of 39a

_N-Benzoyl-4-ethylaniline_ (225 mg, 1.0 mmol) was placed in a 300 mL jacketed beaker, dissolved in 5% H₂O:CH₃OH solution (200 mL) and cooled to -15 °C. Lithium perchlorate trihydrate (4.0 g) and freshly ground NaHCO₃ (6.0 g) were then added and the mixture anodically oxidized at a constant current of 0.15 A (6 - 7 V). The reaction was monitored by TLC (2% EtOAc/CH₂Cl₂ used as eluant) and complete after 35 minutes. The reaction mixture was filtered through a plug of glass wool, H₂O (100 mL) added, and extractive workup with CH₂Cl₂ (3 x 50 mL) gave the crude product as a yellow oil (250 mg). Purification was performed by column chromatography using a Florisil support (10 cm x 2 cm column) and 15% EtOAc/H as eluant. The product 39a (R₁ = 0.43, 2% EtOAc/CH₂Cl₂) was isolated off the column as a light yellow oil (148 mg, 58% yield) with 95% purity. IR (neat) 1670, 1654, 1599, 1246, 1075, 715 cm⁻¹; ¹H NMR δ 7.9 (d, J = 8.0 Hz, 2 H), 7.5 - 7.3 (m, 3 H), 6.4 (AB q, d = 23 Hz, J = 10 Hz, 4 H), 3.1 (s, 3 H), 1.7 (q, J = 7 Hz, 2 H), 0.8 (t, J = 7.0 Hz, 3 H); ¹³C NMR δ 181, 156, 146, 133, 133, 129, 128, 128, 127, 76, 53, 32, 8; HRMS Calcd for C₁₆H₁₇O₂N: m/ë 255.1255, obsd 255.1231.
Preparation of 40a

*N*-Benzoyl-4-sec-butylaniline (253 mg, 1.0 mmol) was placed in a 300 mL jacketed beaker, dissolved in a 5% H$_2$O:CH$_3$OH solution (200 mL) and cooled to -15 °C. Lithium perchlorate trihydrate (4.0 g) and freshly ground NaHCO$_3$ (6.0 g) were then added and the mixture anodically oxidized at a constant current of 0.15 A (7 - 8 V). The reaction was monitored by TLC (2% EtOAc/CH$_2$Cl$_2$ used as eluant) and complete after 35 minutes. The reaction mixture was then filtered through a plug of glass wool, H$_2$O (200 mL) added, and extractive workup with CH$_2$Cl$_2$ (3 x 50 mL) gave the crude product as a yellow oil (270 mg). Purification was performed with a Florisil support (15 cm x 2 cm column, 15% EtOAc/H used as eluant) and Et$_3$N (5 mL) flushed through the column prior to addition of material. The product 40a (R$_f$ = 0.45, 2% EtOAc/CH$_2$Cl$_2$) was isolated as a light yellow oil (128 mg, 45% yield) with 95% purity. IR (neat) 1655, 1599, 1247, 1075, 715 cm$^{-1}$; $^1$H NMR $\delta$ 7.9 (d, $J = 8.0$ Hz, 2 H), 7.5 - 7.3 (m, 3 H), 6.4 (AB q, $dv = 29$ Hz, $J = 10$ Hz, 4 H), 3.1 (s, 3 H), 1.6 - 1.5 (m, 1 H), 0.9 - 0.8 (m, 8 H); $^{13}$C NMR $\delta$ 181, 156, 146, 146, 133, 133, 129, 128, 128, 78, 52, 44, 24, 14, 13; HRMS Calcd for C$_{18}$H$_{21}$O$_2$N: $m/e$ 283.1567, obsd 283.1586.
Preparation of 41a

N-Benzoyl-4-aminobiphenyl (1g, 3.66 mmol), LiClO₄ (4.0g) and NaHCO₃ (4.0g) were added to 5% H₂O : MeOH solution (300 mL) and the mixture stirred vigorously for 20 min. The mixture was then anodically oxidized at a constant current of 0.3 A (6-7 V) at room temperature. The reaction was monitored by TLC (2% EtOAc/CH₂Cl₂ used as eluant) and complete after 79 min (50 % current efficiency). Filtration to remove insoluble NaHCO₃, addition of H₂O (100 mL), and extractive workup with CH₂Cl₂ (3 x 75 mL) gave the crude product as a yellow oil (1.2 g). The oil was dissolved in CH₂Cl₂ (10 mL) and impregnated on Florisil (3.0 g). Column chromatography was performed with a Florisil support (10 cm x 2 cm column, 20% EtOAc/H used as eluant) and Et₃N (2 ml) flushed through the column prior to addition of material. The product was isolated as a light yellow solid (880 mg, 80% yield), mp 88.0 - 90.0 °C. Analytically pure material was obtained with two recrystallizations (Et₂O/H) as a white crystalline solid, mp 93.0 - 93.5 °C. IR (KBr) 1665, 1655, 1610, 1590, 1440, 1235, 1170, 1085, 1070, 1055, 1000, 830, 745, 730, 705, 690 cm⁻¹; ¹H NMR δ 7.97 (d, 7 Hz, 2H), 7.6-7.3 (m, 8H), 6.52 (AB q, dv = 14 Hz, J = 10 Hz, 4 H), 3.36, (s, 3H); ¹³C NMR δ 181, 156, 146, 140, 138, 137, 130, 129, 128, 126, 125, 53; Anal. calcd for C₂₀H₁₇O₂N: C, 79.18; H, 5.65. Found: C, 79.20; H, 5.66.
Preparation of 42a

N-Acetyl-4-aminobiphenyl (0.25 mg, 1.0 mmol, Rf = 0.10, 20% EtOAc/H) and LiClO₄ (2.0 g) were dissolved in 5% H₂O:MeOH (200 mL) and cooled to 0 °C. NaHCO₃ (2.0 g) was then added and the mixture anodically oxidized at a constant current of 0.15 A (3-4.0 V). The reaction was followed by TLC (20% EtOAc/H used as eluent). After 42 min (51% current efficiency) the reaction was complete. The reaction mixture was then filtered via a Buchner funnel to remove insoluble NaHCO₃ and H₂O (50 mL) added to the filtrate. Extractive work-up (CH₂Cl₂, 3 x 75 mL) gave a yellow oil (260 mg). The oil was dissolved in a minimum amount of CH₂Cl₂ and impregnated on Florisil (1.0 g). Column chromatography was then performed using a Florisil support (10 cm x 1 cm column) and Et₂N flushed through the column prior to addition of material. A 25% EtOAc/H solution was used as eluent with a flow rate of approximately 4 mL/min. The first compound isolated off the column gave 4-methoxy-4-phenyl-2,5-cyclohexadienone (Rf = 0.32, 20% EtOAc/H) as a light yellow solid (20 mg, 10% yield), mp 88.0 - 90.0 °C (lit mp 60 91.0 - 92.5 °C) followed by isolation of a light yellow oil (138 mg, 48% yield) characterized as 42a (Rf = 0.24, 20% EtOAc/H). IR (neat) 2930 (w), 1690, 1660, 1610, 1450, 1355, 1210, 1080, 1065, 820, 750, 690 cm⁻¹; ¹H NMR δ 7.4-7.3 (m, 5H), 6.47 (AB q, dv = 14 Hz, J = 10 Hz, 4 H), 3.36 (s, 3H), 2.28 (s, 3H); ¹³C NMR δ
186, 151, 146, 140, 130, 128, 126, 125, 53, 26; Mass spectrum, exact mass calcd for \( \text{C}_{15}\text{H}_{15}\text{O}_{2}\text{N} \) \( m/e \) 241.1099, obsd 241.1088.

**Preparation of 43a**

\( N \)-Benzoyl-2-aminofluorene (250 mg, 0.793 mmol, \( R_f = 0.54 \), 2% EtOAc/\( \text{CH}_2\text{Cl}_2 \)) was added to a solution of 5% \( \text{H}_2\text{O}:\text{MeOH} \) (200 ml) and stirred vigorously for 10 min at room temperature. Lithium perchlorate trihydrate (2.0 g) and \( \text{NaHCO}_3 \) (2.0 g) were added and the reaction mixture anodically oxidized with a constant current of 0.15 A (5-6 V) at room temperature. The reaction was followed by TLC (2% EtOAc/\( \text{CH}_2\text{Cl}_2 \) used as eluent) and judged complete after 25 min (70% current efficiency). \( \text{H}_2\text{O} \) (50 mL) was added to the reaction mixture followed by extraction with \( \text{CH}_2\text{Cl}_2 \) (2 x 75 mL). Removal of solvent in vacuo gave a viscous yellow oil (270 mg). The oil was then dissolved in a minimum amount of \( \text{CH}_2\text{Cl}_2 \) (5 mL) and impregnated on Florisil (1.0 g). Column chromatography was then performed with a Florisil support (10 cm x 1 cm column) and \( \text{Et}_3\text{N} \) (1 mL) flushed through the column prior to addition of material. A 20% EtOAc/H solution was used as eluant with a flow rate of 4 mL/min. The product was the most non-polar spot via TLC (\( R_f = 0.22 \), 2% EtOAc/\( \text{CH}_2\text{Cl}_2 \)) and came off almost immediately as a yellow solution. Removal of solvent in vacuo gave a yellow oil (211 mg). Trituration of the oil with cold ether gave a yellow solid (15
mg), mp 198-201 °C and was identical to starting material by TLC. The mother liquors were combined to give a yellow oil (196 mg, 71%) which was characterized as 43a (Rf = 0.45, 2% EtOAc/CH₂Cl₂) greater than 95% pure by ¹H NMR (200 MHz). IR (neat) 1655, 1615, 1590, 1575, 1240, 1040 cm⁻¹; ¹H NMR δ 7.9 (dd, J = 8 Hz, 1.5 Hz, 2H), 7.6-7.3 (m, 7H), 6.9 (d, Jₐb = 10 Hz, 1H), 6.6 (dd, J = 18 Hz, 2 Hz, 1H), d, J = 2 Hz, 1H), 4.0 (dd, J = 18, 2 Hz, 1H), 3.4 (d, J = 18 Hz, 1H), 3.2 (s, 3H); ¹³C NMR δ 180, 158, 157, 142, 141, 138, 133, 133, 131, 130, 129, 128, 126, 123, 121, 79, 52, 36; Mass spectrum, exact mass calcd for C₂₁H₁₇O₂N m/e 315.1255, obsd 315.1238.

Preparation of 44a

N-Acetyl-2-aminofluorene (0.2g, 0.790 mmol, Rf = 0.23, 40% EtOAc/H) and LiClO₄ (2.0 g) were dissolved in 5% H₂O:MeOH (200 mL) and cooled to 0 °C. NaHCO₃ (2.0 g) was added and the mixture anodically oxidized at a constant current of 0.3 A (6-7 V).

The reaction was followed by TLC (40% EtOAc/H used as eluent) and found to be complete after 0.5 h (60 % current efficiency). The reaction mixture was then filtered through a plug of glass wool to remove insoluble NaHCO₃ and H₂O (100 mL) added to the filtrate. Extraction was performed with CH₂Cl₂ (3 x 70 mL), the organic phase separated, dried through Na₂SO₄, and the solvent removed in vacuo giving a yellow-brown oil (215 mg). The oil was dissolved in a
minimum amount of CH$_2$Cl$_2$ and impregnated on Florisil (1.0 g).
Column chromatography was performed using a Florisil support (10 cm x 2 cm column) and Et$_3$N (1 mL) flushed through the column prior to addition of compound. A 25% EtOAc/H eluent was used with a flow rate of approximately 2 mL/min. Compound 44c ($R_f$ = 0.59, 40% EtOAc/H) was isolated as a light yellow solid (20 mg, 12% yield), mp 99.0 - 102.0 °C and spectral properties corresponding with authentic sample (see Experimental in Chapter 3). Compound 44a ($R_f$ = 0.51, 40% EtOAc/H) was isolated as a yellow oil (97 mg, 43% yield) which was 95% pure by $^1$H NMR (200 MHz). IR (neat) 2920 (w), 1695, 1670, 1605, 1220, 1045, 750 cm$^{-1}$; $^1$H NMR δ 7.43-7.33 (m, 1 H), 7.32-7.26 (m, 3 H), 6.93 (d, $J$ = 10 Hz, 1 H), 6.52 (dd, $J$ = 10 Hz, 2 Hz, 1 H), 6.35 (d, $J$ = 2 Hz, 1 H), 3.98 (dd, $J$ = 18 Hz, 2 Hz, 1 H), 3.45 (d, $J$ = 18 Hz, 1 H), 3.12 (s, 3 H), 2.23 (s, 3 H); HRMS Calcd for C$_{16}$H$_{15}$O$_2$N: m/e 253.1099, obsd 253.1068.

Preparation of 45a

$N$-Benzoyl-5-aminoindane (237 mg, 1.0 mmol, $R_f$ = 0.61, 2% EtOAc/CH$_2$Cl$_2$) was placed in a 300 mL jacketed beaker, dissolved in 5% H$_2$O:CH$_3$OH solution (200 mL) and cooled to -15 °C. Lithium perchlorate trihydrate (4.0 g) and freshly ground NaHCO$_3$ (4.0 g) were added and the mixture anodically oxidized at a constant current of 0.15 A (4 - 5 V). The reaction was
monitored by TLC (2% EtOAc/CH₂Cl₂ used as eluant) and complete after 35 minutes. H₂O (100 mL) was added and extractive workup performed with CH₂Cl₂ (2 x 150 mL) giving the crude product as a yellow oil (250 mg). The oil was impregnated on Florisil (2.0 g) and column chromatography performed with a Florisil support (10 cm x 2 cm column) using 20% EtOAc/H as eluant. The product 45a (Rf = 0.39, 2% EtOAc/CH₂Cl₂) was isolated as a light yellow oil (113 mg, 45% yield) with absolute purity by ¹H NMR. IR (neat) 1660, 1598, 1246, 1173, 1111, 1090, 1056 cm⁻¹; ¹H NMR δ 7.9 (d, J = 8.0 Hz, 2 H), 7.6 - 7.4 (m, 3 H), 6.5 (AB q, lower field ¹H a doublet, Δv = 13 Hz, J = 10, 1.5 Hz, 2 H), 6.3 (d, J = 1.5 Hz, 1 H), 3.0 (s, 3 H), 2.5 (m, 2 H), 2.2 (m, 2 H), 1.7 (m, 2 H); ¹³C NMR δ 180, 162, 156, 141, 133, 133, 129, 129, 128, 120, 79, 51, 36, 29, 21; HRMS Calcd for C₁₇H₁₇O₂N: m/z 267.1255, obsd 267.1295.

**Anodic Oxidation Studies (Table 6)**

**Preparation of 34b**

**Entry 2.** N-Benzoyl-4-aminotoluene (216 mg, 1.0 mmol) was placed in a 300 mL beaker, dissolved in a 30% H₂O:CH₃CN solution (200 mL), followed by the addition of freshly ground NaHCO₃ (6.0 g) and lithium perchlorate trihydrate (4.0 g). The
mixture was then anodically oxidized at a constant current of 0.15 A (4 - 5V) at room temperature. The reaction was monitored by TLC (40% EtOAc/H used as eluant) and complete after 35 minutes. The reaction mixture was then filtered through a plug of glass wool, H₂O (200 mL) added and extraction performed with CH₂Cl₂ (2 x 75 mL). The organic layer was washed with brine (100 mL), and dried through CaSO₄. Removal of solvent in vacuo gave a yellow oil (220 mg). Column chromatography was then performed with a Florisil support (10 cm x 1 cm column) and 25% EtOAc/H used as eluant. The product 34b (Rf = 0.28, 40% EtOAc/H) was isolated as a light yellow oil (100 mg, 44% yield) with 95% purity. IR (neat) 3388, 1653, 1599, 1580, 1449, 1313, 1248, 1079, 1063, 838, 724 cm⁻¹; ¹H NMR δ 7.9 (d, J = 8.0 Hz, 2 H), 7.5 - 7.3 (m, 3 H), 6.3 (AB q, δv = 77 Hz, J = 10 Hz, 4 H), 3.5 (bs, 1 H), 1.4 (s, 3 H); ¹³C NMR δ 181, 156, 148, 133, 132, 129, 128, 123, 67, 27; HRMS Calcd for C₁₄H₁₃O₂N: m/e 227.0943, obsd 227.0937.

Entry 7. *N*-Benzoyl-4-aminotoluene (215 mg, 1.0 mmol) was dissolved in 30% H₂O:THF (200 mL), followed by the addition of lithium perchlorate trihydrate (4 g) and freshly ground NaHCO₃ (6 g). The mixture was electrolyzed at a constant current of 0.15 A and monitored by TLC (2% EtOAc/CH₂Cl₂). After 45 minutes (theoretical time = 21 minutes) the oxidation was discontinued and extractive workup performed with CH₂Cl₂ (2 x 50 mL) giving a yellow oily solid (230 mg) as crude product. The solid was impregnated on
Florisil (2 g) and column chromatography performed with a Florisil support (10 cm x 2 cm column) using 20% EtOAc/H as eluant. The first component was isolated as white solid (110 mg) and characterized as starting amide 33 (51% recovered yield), mp 151.0 - 152.0 °C (lit57 mp 157.0 - 158.0 °C). The second and third components were isolated as yellow oils and characterized as compounds 34b (28 mg, 12% yield) and 35 (20 mg, 9% yield).

**Entry 8.** N-Benzoyl-4-aminotoluene (215 mg, 1.0 mmol) was dissolved in 30% H$_2$O:DMF (200 mL), followed by the addition of lithium perchlorate trihydrate (4 g) and freshly ground NaHCO$_3$ (6 g). The mixture was electrolyzed at a constant current of 0.15 A and monitored by TLC (2% EtOAc/CH$_2$Cl$_2$). After 45 minutes the oxidation was discontinued and extractive workup performed with CH$_2$Cl$_2$ (2 x 50 mL) giving a yellow oil (240 mg) as crude product. The oil was impregnated on Florisil (1.5 g) and column chromatography performed with a Florisil support (10 cm x 2 cm column) using 20% EtOAc/H as eluant. The first component was isolated as a white solid (53 mg) and characterized as starting amide 33 (25% recovered yield), mp 151.0 - 152.0 °C. The second and third components were isolated as yellow oils and characterized as compounds 34b (50 mg, 22% yield) and 35 (34 mg, 16% yield).
Preparation of 38b

*N*-Carbonylmethoxy-4-aminotoluene (83 mg, 0.5 mmol, *R* = 0.80, 40% EtOAc/H) was dissolved in a 30% H$_2$O : CH$_3$CN solution (100 mL) followed by the addition of lithium perchlorate trihydrate (2.0 g) and freshly ground NaHCO$_3$ (2.0 g). The mixture was then anodically oxidized at a constant current of 0.15 A (4 - 5 V) at room temperature for 13 minutes. Water (50 mL) was added and extractive workup performed with CH$_2$Cl$_2$ (2 x 75 mL) giving a yellow oil (100 mg). Column chromatography was performed with a Florisil support (10 cm x 2 cm column) and 25% EtOAc/H used as eluant. The product 38b (*R* = 0.28, 40% EtOAc/H) was isolated as a light yellow oil (35 mg, 38% yield) which was 95% pure by $^1$H NMR. IR (neat) 3322, 1706, 1542, 1515, 1456, 1275, 1240, 1115 cm$^{-1}$; $^1$H NMR $\delta$ 6.4 (AB q, $\Delta$v = 78 Hz, J = 10 Hz, 4 H), 3.8 (s, 3 H), 1.4 (s, 3 H); HRMS Calcd for C$_9$H$_{11}$O$_3$N: m/e 181.0736, obsd 181.0744.

Preparation of 39b

*N*-Benzoyl-4-ethylaniline (225 mg, 1.0 mmol) was placed in a 300 mL beaker, dissolved in a 30% H$_2$O:CH$_3$CN (200 mL) solution, followed by the addition of freshly ground NaHCO$_3$ (6.0 g) and lithium perchlorate trihydrate (4.0 g). The
mixture was then anodically oxidized at a constant current of 0.15 A (4 - 5V) at room temperature. The reaction was monitored by TLC (40% EtOAc/H used as eluant) and complete after 35 minutes. The reaction mixture was then filtered through a plug of glass wool, H₂O (200 mL) added and extraction performed with CH₂Cl₂ (75 mL). The organic layer was washed with brine (100 mL), and dried through CaSO₄. Removal of solvent in vacuo gave a yellow oil (240 mg). The oil was dissolved in CH₂Cl₂ (5 mL), Et₃N (3 mL) added and impregnated on Florisil (2.0 g). Column chromatography was then performed with a Florisil support (10 cm x 1 cm column, 25% EtOAc/H used as eluant) and Et₃N (5 mL) flushed through the column prior to addition of material. The first component eluted off the column was isolated as a light yellow oil (71 mg, 30% yield) with approximately 95% purity. IR (neat) 3385, 1643, 1599, 1313, 1257, 1062, 1023, 718 cm⁻¹; ¹H NMR δ 7.9 (d, J = 8.0 Hz, 2 H), 7.5 - 7.3 (m, 3 H), 6.4 (AB q, Δv = 43 Hz, J = 10 Hz, 4 H), 1.7 (q, J = 7.0 Hz, 2 H), 0.9 (t, J = 7.0 Hz, 3 H); ¹³C NMR δ 181, 156, 147, 133, 132, 129, 128, 125, 70, 33, 8; HRMS Calcd for C₁₅H₁₅O₂N: m/e 241.1099, obsd 241.1103.

Anodic oxidation of N-Benzoyl-4-sec-butylaniline 40.

N-Benzoyl-4-sec-butylaniline (253 mg, 1.0 mmol) was placed in a 300 mL beaker, dissolved in a 30% H₂O:CH₃CN solution (200 mL), followed by the addition of freshly ground NaHCO₃ (6.0 g) and lithium perchlorate trihydrate (4.0 g). The mixture was then
anodically oxidized at a constant current of 0.15 A (4 - 5 V) at room temperature. The reaction was monitored by TLC (40% EtOAc/H used as eluant) and complete after 40 minutes. The reaction mixture was then filtered through a plug of glass wool, H₂O (200 mL) added, and extraction performed with CH₂Cl₂ (2 x 75 mL). The organic layer was washed with brine (100 mL), and dried through CaSO₄. Removal of solvent in vacuo gave a yellow oil (275 mg). The oil was dissolved in CH₂Cl₂ (5 mL), Et₃N (3 mL) added, and impregnated on Florisil (2.0 g). Column chromatography was then performed with a Florisil support (10 cm x 1 cm column, 25% EtOAc/H used as eluant) and Et₃N (5 mL) flushed through the column prior to addition of material. The product, 4-(N-benzoylamino)-6-sec-butylphenol 40c was isolated as a light red oil (126 mg, 47% yield) and had spectral properties identical with authentic sample (see Preparation of 40c).

**Preparation of 41b**

*N-Benzoyl-4-aminobiphenyl* (500 mg, 1.84 mmol, Rₜ = 0.45, 25% EtOAc/H) was dissolved in DMF (30 mL), and added to 10% H₂O : CH₃CN solution (300 mL). Lithium perchlorate trihydrate (4.0 g) and NaHCO₃ (4.0 g) were added and the mixture anodically oxidized at a constant current of 0.15 A (6-7 V) at room temperature. The reaction was monitored by TLC (25% EtOAc/H
solution used as eluent) and complete after 1h 30 min (44% current efficiency). Filtration to remove insoluble NaHCO₃, addition of H₂O (100 mL), extraction with CH₂Cl₂ (3 x 150 mL) and brine wash (2 x 75 mL) gave the crude product as a brown residue (490 mg). The residue was dissolved in CH₂Cl₂ (10 mL), Et₃N (5 mL) added, and impregnated on Florisil (2 g). Column chromatography was performed with a Florisil support (15 cm x 2 cm column, 25% EtOAc/H used as eluant) and Et₃N (5 mL) flushed through the column prior to addition of material. The product 41b (Rₐ = 0.35, 25% EtOAc/H) was isolated as a light yellow oil which solidified with addition of cold ether and scratching with a spatula (170 mg), mp 105.0 - 107.0 °C. Recrystallization from Et₂O/H gave a pale white solid (156 mg, 30% yield), mp 113.0 - 115.0 °C. Analytically pure material was obtained with an additional recrystallization (Et₂O/H) giving a white crystalline solid, mp 118.5 - 119.0 °C. A second compound was isolated as a light yellow solid (80 mg, 15% yield) and characterized as 41c (Rₐ = 0.20, 25% EtOAc/H). The spectral properties of 41b are described as follows (see Preparation of 41c for spectral properties): N-Benzoyl-4-Hydroxy-4-Phenyl-p-Benzoquinone

**Imine 41b.** IR (KBr) 3340, 1665, 1635, 1610, 1580, 1450, 1265, 1045, 945 cm⁻¹; ¹H NMR (d₆-DMSO) δ 7.87 (dd, J = 8, 1.5 Hz, 2H), 7.7-7.6 (m, 1H), 7.6-7.5 (m, 2H), 7.4-7.3 (m, 5H), 6.47 (AB q, Δν = 98 Hz, J = 10 Hz, 4 H), 6.43 (s, 1H); ¹³C NMR (d₆-DMSO) δ 180, 157, 150, 142, 135, 134, 130, 129, 128, 126, 123, 71; Anal. calcd for C₁₉H₁₅O₂N: C, 78.87; H, 5.22. Found: C, 78.67; H, 5.22.
Preparation of 45b.

*N*-Benzoyl-5-aminoindane (237 mg, 1.0 mmol), lithium perchlorate trihydrate (4.0 g) and freshly ground NaHCO₃ (4.0 g) were added to a 30% H₂O : CH₃CN solution (200 mL), the mixture stirred vigorously for 10 minutes, and anodically oxidized at a constant current of 0.15 A (4 - 5 V) at room temperature. The reaction was monitored by TLC (40% EtOAc/H used as eluant) and complete after 40 minutes. H₂O (100 mL) was then added, extraction performed with CH₂Cl₂ (2 x 150 mL), the organic layer separated and washed with brine (100 mL) and the solvent removed in vacuo giving the crude product as a brown oil (250 mg). The oil was impregnated on Florisil (2.0 g) and column chromatography performed with a Florisil support (15 cm x 2 cm column, 25% EtOAc/H used as eluant) and Et₃N (5 mL) flushed through the column prior to addition of material. The product 45b (Rf - 0.61, 40% EtOAc/H) was isolated as a yellow oil (80 mg, 32% yield) with 90% purity by ¹H NMR. IR (neat) 3374, 1668, 1599, 1248, cm⁻¹; ¹H NMR δ 7.9 (d, J = 8.0 Hz, 2 H), 7.6 - 7.4 (m, 3 H), 6.6 (AB q, with lower field ¹H as dd, Δν = 98 Hz, J = 10 Hz, 1.5 Hz, 2 H), 6.0 (d, J = 1.5 Hz, 1 H), 2.8 (m, 1 H), 2.8 - 2.6 (s, 1 H), 2.4 - 2.3 (m, 1 H), 2.2 - 2.0 (m, 2 H), 2.0 - 1.8 (m, 1 H), 1.6 - 1.5 (m, 1 H); HRMS Calcd for C₁₆H₁₅O₂N: m/e 253.1099, obsd 253.1094.
Preparation of 40d.

2-sec-Butylphenol (2.0 g, 13.0 mmol) was placed in a 100 mL three-neck round-bottom flask, dissolved in glacial acetic acid (15 mL) and equipped with an addition funnel. The solution was cooled to 10 °C and a solution of concentrated nitric acid (820 mg, 13.0 mmol) and glacial acetic acid (5 mL) added slowly with vigorous stirring of the solution. Upon complete addition of the nitric acid, the solution was warmed to room temperature and stirred for 1.0 h. The reaction mixture was then neutralized with solid NaHCO₃ (20 g), H₂O (200 mL) added and extractive work-up performed with CH₂Cl₂ (3 x 100 mL) to give a brown viscous oil (2.8 g). Column chromatography was performed using flash silica gel (15 cm x 3 cm column) and 10% EtOAc/H used as eluant. The first component off the column was 2-nitro-6-sec-butylphenol (Rₚ = 0.9, 20% EtOAc/H) isolated as a lime green oil (1.0 g, 40% yield). The second component was 4-nitro-6-sec-butylphenol (Rₚ = 0.53, 20% EtOAc/H) isolated as a light red solid (1.3 g, 51% yield), mp 75.0 - 76.0 (lit mp 61, 75.0 - 77.0 °C). The spectral data are as follows: 2-nitro-6-sec-butylphenol, IR (neat) 3181, 2964, 1540, 1457, 1448 cm⁻¹; H NMR δ 11.0 (s, 1 H), 7.9 (dd, J = 8.0, 1.5 Hz, 1 H), 7.4 (dd, J = 8.0, 1.5 Hz, 1 H), 6.9 (t, J = 8.0 Hz, 1 H), 3.2 (sextet, J = 7.0 Hz, 1 H), 1.6 (pentet, J
86 Hz, 2 H), 1.2 (d, J = 7.0 Hz, 3 H), 0.9 (t, J = 7.0 Hz, 3 H);

4-nitro-6-sec-butylphenol, IR (KBr) 3359, 1587, 1512, 1483, 1333,
1277, 1218 cm⁻¹; ¹H NMR δ 8.0 (d, J = 2.0 Hz, 1 H), 7.9 (dd, J =
8.0, 2.0 Hz, 1 H), 6.8 (d, J = 8.0 Hz, 1 H), 6.3 (bs, 1 H), 3.0
(sextet, J = 7.0 Hz, 1 H), 1.6 (pentet, J = 7.0 Hz, 2 H), 1.2 (d, J
= 7.0 Hz, 3 H), 0.9 (t, J = 7.0 Hz, 3 H).

Preparation of 40c.

4-Nitro-2-sec-butylphenol (1.3 g, 6.7
mmol) was placed in a 250 mL Paar
hydrogenation bottle, dissolved in THF (100
mL), and 10% Pt/C catalyst (100 mg) added.
The contents were then placed under
hydrogen (70 psi) for 12 h. The solution
was then filtered through celite, Et₃N (1.0 mL, 10.0 mmol) added to
the filtrate followed by the slow addition of benzoyl chloride (0.8
mL, 10.0 mmol) causing immediate formation of precipitate. The
mixture was placed on a steam bath and heated to boiling for 15.0
minutes. The precipitate was filtered off, washed with THF (20 mL),
and the solvent removed in vacuo to give a brown viscous oil (1.6
g). Column chromatography was then performed with flash silica gel
(8 cm x 3 cm column) and EtOAc used as eluant. The product 40c (Rᵣ
= 0.17, 20% EtOAc/H) was isolated as a light purple oil (1.1 g, 61%
yield). IR (neat) 3313, 1643, 1612, 1544, 1507, 1430, 1265 cm⁻¹;
¹H NMR δ 8.1 (s, 1 H), 7.8 (dd, J = 8.0 Hz, 2 H), 7.5 - 7.2 (m, 5
H), 6.7 (d, J = 8.0 Hz, 1 H), 3.0 (sextet, J = 7.0 Hz, 1 H), 1.5
(pentet, J = 7.0 Hz, 2 H), 1.2 (d, J = 7.0 Hz, 3 H), 0.9 (t, J =
7.0 Hz, 3 H); HRMS Calcd for C\textsubscript{17}H\textsubscript{19}O\textsubscript{2}N: m/e 269.1411, obsd 269.1414.

**Preparation of 41d.**

2-Hydroxy-biphenyl (1.0 g, 5.9 mmol) was dissolved in glacial acetic acid (30 mL) in a 250 mL 3-neck round-bottom flask equipped with addition funnel. The solution was then cooled in an ice-bath to 10 °C which became heterogeneous. A solution of concentrated HNO\textsubscript{3} (0.34 mL, 5.9 mmol) and glacial acetic acid (5.0 mL) was then added slowly via addition funnel to the heterogeneous mixture turning a bright orange color. Upon completion, the ice-bath was removed and the solution stirred vigorously at room temperature for 2.0 h. Saturated NaHCO\textsubscript{3} (200 mL) was then added, followed by extractive work-up with CH\textsubscript{2}Cl\textsubscript{2} (3 x 150 mL) giving a bright yellow sticky solid (1.2 g). Column chromatography was then performed with flash silica gel (15 cm x 1 cm column) using 10 % EtOAc/H as eluant. The first compound off the column was 2-hydroxy-3-nitrobiphenyl (R\textsubscript{f} = 0.63, 20% EtOAc/H) as a bright yellow solid (700 mg, 60 % yield), mp 60.0 - 61.0 °C (lit\textsuperscript{62} mp 61.0 - 62.0 °C). The second compound isolated off the column was 2-hydroxy-5-nitro-biphenyl (R\textsubscript{f} = 0.25, 20% EtOAc/H) as a bright yellow solid (400 mg, 40 % yield), mp 124.0 - 125.0 °C (lit\textsuperscript{62} mp
124.0 - 125.0 °C). The spectral properties follow:

2-Hydroxy-3-Nitrobiphenyl. $^1$H NMR $\delta$ 11.1 (s, 1 H), 8.1 (d, $J = 8.0$ Hz, 1 H), 7.6-7.3 (m, 6 H), 7.0 (t, $J = 8.0$ Hz, 1 H);

2-Hydroxy-5-Nitrobiphenyl. $^1$H NMR $\delta$ 8.2-8.1 (m, 2 H), 7.5-7.3 (m, 5 H), 7.0 (d, $J = 8.0$ Hz, 1 H).

Preparation of 41c.

2-Hydroxy-4-nitrobiphenyl (300 mg, 1.4 mmol) was placed in a 250 mL Paar hydrogenation bottle and completely dissolved in THF (40 mL). A 10% Pt/C catalyst (25 mg) was added and the contents placed under hydrogen (65 psi) for 3.0 h at room temperature. The mixture was then filtered through celite and the filtrate placed in a 1 L Erlenmeyer flask with THF (100 mL). Et$_3$N (0.2 mL, 1.5 mmol) was added followed by the slow addition of a solution of benzoyl chloride (0.2 mL, 1.5 mmol) in THF (10 mL). Mild exothermicity and precipitation of a white solid was observed. The mixture was heated on a steam bath to boiling for 15 minutes with intermittent swirling of the solution. After cooling to room temperature, cold H$_2$O (300 mL) was added to the mixture causing precipitation of a white solid. The solid was filtered and washed twice with cold H$_2$O (10 mL) and dried under reduced vacuum (0.2 torr) giving a white solid (250 mg, 62% yield); mp 188.0 - 189.0 °C. IR (KBr) 3250, 1645, 1625, 1530, 1505, 1490, 1410, 690 cm$^{-1}$; $^1$H NMR
(d$_6$-DMSO) 10.1 (s, 1 H), 9.5 (s, 1 H), 7.9 (d, J = 7.0 Hz, 2 H), 7.6 (d, J = 2.0 Hz, 1 H), 7.5 - 7.3 (m, 9 H), 6.9 (d, J = 9.0 Hz, 1 H); HRMS Calcd for C$_{19}$H$_{15}$O$_2$N: m/e 289.1099, obsd 289.1095.

Anodic Oxidation of 46 in 30% H$_2$O:CH$_3$CN.

N-Acetyl-5-aminoindane (350 mg, 2.0 mmol) was added to a 30% H$_2$O:CH$_3$CN solution (200 mL) and stirred vigorously until completely homogeneous. Lithium perchlorate trihydrate (4.0 g) and freshly ground NaHCO$_3$ were added and the mixture electrolyzed at a constant current of 0.15 A (4 - 5 V) at room temperature. The reaction was monitored by TLC (50% EtOAc/H) and complete after 1 h 20 minutes. Extractive workup was performed with CH$_2$Cl$_2$ (2 x 75 mL) giving a brown oil (375 mg) as crude product. The oil was impregnated on Florisil (2.0 g) and column chromatography performed with a Florisil support (10 cm x 2 cm, 40% EtOAc/H used as eluant) and Et$_3$N (5 mL) flushed through the column prior to addition of material. The first component (R$_f$ = 0.67, 50% EtOAc/H) eluted from the column was isolated as a yellow oil (30 mg) which was a mixture by $^1$H NMR (200 MHz) of 46b (17 mg, 4% yield) and 46c (13 mg, 4% yield).

Anodic Oxidation of 43 in 1:1:8 H$_2$O:DMF:CH$_3$CN.

N-Benzoyl-2-aminofluorene (100 mg, R$_f$ = 0.46, 25% EtOAc/H, 0.35 mmol) was dissolved in DMF (8 mL) and placed in the anode compartment of a divided cell.
electrochemical apparatus. Lithium perchlorate trihydrate was dissolved in 10% H$_2$O:CH$_3$CN solution (150 mL) and added to the cell. The cell was then submersed in an ice bath and cooled to 2 °C after 15 minutes. Freshly ground NaHCO$_3$ was added to the anode compartment of the cell and the mixture anodically oxidized at a constant current of 0.15 A (20 - 25 V). The oxidation was followed by TLC (25% EtOAc/H) and determined complete after 25 minutes. The reaction mixture in the anode compartment was removed, filtered through a plug of glass wool, and H$_2$O added (100 mL). Prior to extraction, all glassware in potential contact with material was washed with Et$_3$N. Extraction was performed with CH$_2$Cl$_2$ (3 x 75 mL), the organic layer washed with brine (100 mL) and filtered slowly through Na$_2$SO$_4$. Removal of solvent in vacuo gave a brown liquid which was placed under vacuum (0.2 torr) to remove excess DMF giving a brown oily solid (112 mg). The solid was impregnated on Florisil (1.0 g) and column chromatography performed with a Florisil support (15 cm x 2 cm column, 25% EtOAc/H used as eluant) and Et$_3$N (5 mL) flushed through the column prior to addition of material. The product 43b (R$_f$ = 0.16, 25% EtOAc/H) was isolated as a yellow oil (40 mg, 38% yield) which was approximately 80% pure by $^1$H NMR with the major impurity being starting amide 43. $^1$H NMR (500 MHz, (CD$_3$)$_2$CO) δ 7.9 (d, J = 8.0 Hz, 2 H), 7.6 - 7.4 (m, 4 H), 7.3 - 7.2 (m, 4 H), 6.4 (dd, J = 10.0, 1.7 Hz, 1 H), 6.2 (d, J = 1.6 Hz, 1 H), 4.1 (dd, J = 16.0, 2.0 Hz, 1 H), 3.6 (d, J = 18.0 Hz, 1 H); $^{13}$C (500 MHz, (CD$_3$)$_2$CO) 181, 158, 142, 142, 134, 131, 131, 130, 129, 128,
Preparation of 1-(2-Hydroxyethoxy)-4-Nitrobenzene, 47a.

4-Nitrophenol (1.0 g, 8.1 mmol) was dissolved in DMF (4 mL), placed in a 25 mL round-bottom flask and equipped with a condenser. Ethylene carbonate (858 mg, 8.2 mmol) and tetrabutylammonium iodide (300 mg, 0.8 mmol) were added and the mixture heated to 130 °C for 22 h. The reaction mixture was cooled to room temperature, H₂O (100 mL) added, and extractive workup performed with CH₂Cl₂ (2 x 50 mL) giving a sticky yellow-brown solid (1.8 g). The solid was then dissolved in CH₂Cl₂ (5 mL) and flushed through a Florisil support (10 cm x 2 cm column) using CH₂Cl₂ as eluant. The product 47a (Rf = 0.43, 40% EtOAc/H) was isolated as a light yellow solid (1.2 g, 80% yield), mp 87.0 - 89.0 °C. IR (KBr) 3269, 1608, 1596, 1507, 1341, 1332, 1270, 1078, 1040, 841 cm⁻¹; ¹H NMR δ 8.2 (d, J = 8.0 Hz, 2 H), 7.0 (d, J = 8.0 Hz, 2 H), 4.3 (t, J = 7.0 Hz, 2 H), 4.1 (t, J = 7.0 Hz, 2 H), 2.5 (bs, 1 H); HRMS Calcd for C₈H₇O₄N: m/e 183.0531, obsd 183.0531.
Preparation of \(N\)-Benzoyl-4-(2-Hydroxyethoxy)aniline 47.

1-(2-Hydroxyethoxy)-4-nitrobenzene (430 mg, 2.4 mmol) was dissolved in THF (50 mL) and placed in a Paar hydrogenation bottle and 5% Pt/C catalyst (40 mg) added. The contents were then placed under hydrogen (60 psi) for 12 h. The reaction mixture was filtered through celite, Et\(_3\)N (0.4 mL, 2.5 mmol) and THF (100 mL) added to the filtrate, followed by the addition of benzoyl chloride (0.33 mL, 2.5 mmol) forming a white precipitate. The mixture was heated to boiling for 10 minutes, cooled to room temperature, and H\(_2\)O (200 mL) added. Extractive workup was performed with CH\(_2\)Cl\(_2\) (2 x 75 mL) giving a sticky yellow solid (710 mg) as crude product. Trituration with cold Et\(_2\)O/H gave a pale white solid (350 mg, 57% yield, \(R_f = 0.13, 40\% \text{ EtOAc/H}\)), mp 156.0 - 158.0 °C. IR (KBr) 3334, 1648, 1534, 1517 cm\(^{-1}\); \(^1\)H NMR ((CH\(_3\))\(_2\)CO) \(\delta 8.0 (dd, J = 7.0, 2.0 \text{ Hz}, 2 \text{ H}), 7.7 (d, J = 9.0 \text{ Hz}, 2 \text{ H}), 7.6 - 7.5 (m, 3 \text{ H}), 7.0 (d, J = 9.0 \text{ Hz}, 2 \text{ H}), 4.1 (t, J = 6.0 \text{ Hz}, 2 \text{ H}), 3.0 (q, J = 6.0 \text{ Hz}, 2 \text{ H}); HRMS Calcd for C\(_{15}\)H\(_{15}\)O\(_3\)N: m/e 257.1052, obsd 257.1047.

Anodic Oxidation of 47 in 5% H\(_2\)O:CH\(_3\)OH

The starting amide 47 (122 mg, 0.47 mmol) was dissolved in 5% H\(_2\)O:CH\(_3\)OH (200 mL) and cooled to 0 °C. Lithium perchlorate trihydrate (4 g) and freshly
ground NaHCO₃ (8 g) were added and the mixture electrolyzed at a constant current of 0.15 A (5 - 6 V). The reaction was monitored by TLC (50% EtOAc/H) and appeared complete after 20 minutes. Extractive workup was performed with CH₂Cl₂ (2 x 50 mL) giving a light yellow oil as crude product. The oil was impregnated on Florisil (1.5 g) and column chromatography performed with a Florisil support (10 cm x 2 cm column) using 25% EtOAc/H as eluant. Compound 49 (Rₜ = 0.42, 40% EtOAc/H) was isolated as a light solid (20 mg, 17% yield), mp 96.0 - 98.0 °C (see Experimental Section in Chapter 4 for spectral properties) followed by isolation of 48 (Rₜ = 0.15, 40% EtOAc) as a clear colorless oil (65 mg, 48% yield). IR (KBr) 3447, 1658, 1603, 1249, 1108, 1060, 1022 cm⁻¹; ¹H NMR δ 7.9 (d, J = 8.0 Hz, 2 H), 7.6 - 7.4 (m, 3 H), 6.5 (AB q, Δv = 20 Hz, J = 10 Hz, 4 H), 3.7 (m, 4 H), 3.4 (s, 3 H); HRMS Calcd for C₁₆H₁₇O₄N: m/e 287.1157, obsd 287.1157.

List of References


49. Taken from ref. 48.


51. 2-Chloronaphthalene was not electrochemically stable under the reaction conditions. Therefore an aliquot (1 mL) of a 20 mM solution of 2-chloronaphthalene was added to an aliquot (1 mL) of reaction mixture prior to HPLC analysis.


53. Ibid, p. 182.


$^1$H NMR Spectra
Figure 10. 500 MHz $^1$H NMR Spectrum of 8
Figure 11. 200 MHz $^1$H NMR Spectrum of 34a
Figure 12. 200 MHz $^1$H NMR Spectrum of 38a
Figure 13. 50 MHz $^{13}$C NMR Spectrum of 38a
Figure 14. 200 MHz $^1$H NMR Spectrum of 39a
Figure 15. 50 MHz $^{13}$C NMR Spectrum of 39a
Figure 16. 200 MHz $^1$H NMR Spectrum of 40a
Figure 18. 200 MHz $^1H$ NMR Spectrum of 41a
Figure 19. 50 MHz $^{13}$C NMR Spectrum of 41a
Figure 20. 200 MHz $^1$H NMR Spectrum of 42a
Figure 21. 50 MHz $^{13}$C NMR Spectrum of 42a
Figure 22. 200 MHz $^1$H NMR Spectrum of 43a
Figure 23. 50 MHz $^{13}$C NMR Spectrum of 43a
Figure 24. 200 MHz $^1$H NMR Spectrum of 44a
Figure 25. 200 MHz $^1$H NMR Spectrum of 45a
Figure 26. 50 MHz $^{13}$C NMR Spectrum of 45a
Figure 27. 200 MHz $^1$H NMR Spectrum of 34b
Figure 28. 50 MHz $^{13}$C NMR Spectrum of 34b
Figure 29. 200 MHz $^1$H NMR Spectrum of 38b
Figure 30. 200 MHz $^1$H NMR Spectrum of 39b
Figure 31. 50 MHz $^{13}$C NMR Spectrum of 39b
Figure 32. 200 MHz $^1$H NMR Spectrum of 41b
Figure 33. 50 MHz $^{13}\text{C}$ NMR Spectrum of 41b
Figure 34. 200 MHz $^1$H NMR Spectrum of 45b
Figure 35. 200 MHz $^1$H NMR Spectrum of 40d
Figure 36. 200 MHz $^1$H NMR Spectrum of 40c
Figure 37. 200 MHz $^1$H NMR Spectrum of 41d
Figure 38. 200 MHz $^1$H NMR Spectrum of 41c
Figure 39. 200 MHz $^1$H NMR Spectrum of 47a
Figure 40. 200 MHz $^1$H NMR Spectrum of 47
Figure 41. 200 MHz $^1$H NMR Spectrum of 48
Figure 42. 200 MHz $^1$H NMR Spectrum of o-sec-butyl-6-nitrophenol
Figure 43. 200 MHz $^1$H NMR Spectrum of o-nitro-6-phenylphenol
Chapter III

The Reactivity of N-Acylated-p-Benzquinol Imines with Aqueous Media and N-Alkyl and N-Aryl Amines.

Results and Discussion

As was discussed in Chapter 1, investigations on the hydrolysis of p-substituted N-(sulfonatooxy)-N-acetyl-arylamines 50 have provided evidence for the formation of the N-acetyl-p-benzoquinol imines 51 (Scheme XXIII). However, intermediates such as 51 have not been available for direct study prior to this investigation. Therefore efforts were directed at investigating the stability and reactivity of these important intermediates in aqueous media and with nucleophiles such as alkyl amines and thiols (Chapter 4).

Scheme XXIII.

\[
\begin{array}{c}
\text{50} \\
\text{H}_2\text{O} \\
\text{51}
\end{array}
\]

In Chapter 1, during the synthesis and isolation of the N-benzoyl-4-hydroxy- and N-benzoyl-4-methoxy-4-phenyl-p-benzoquinol imines 41a and 41b, a sharp contrast in acidic stability of the two
systems was observed. This instability was determined to be an acid-catalyzed dienone-phenol like rearrangement forming the 2-hydroxy- and 2-methoxy-biphenyl systems 40c and 41c. Interestingly, the N-benzoyl-4-hydroxy-4-phenyl-p-benzoquinone imine 41b appeared to favor this pathway under mild acidic conditions much more readily than its methoxy counterpart. Therefore a kinetic study was undertaken to determine the relative rates for 1,2-phenyl migration of compounds 41a and 41b using uv spectroscopy to monitor the reaction.

Inspection of the uv spectra of 41a and 41b and their respective rearrangement products determined the kinetic measurements could not be done at a single wavelength without an overlap of absorbance in the region of 325-220 nm. Therefore the kinetic study was conducted by recording the change in absorbance at two wavelengths and the product concentration calculated using the two equations shown below:

\[
A_{310} = E_s^{310}x_{Cs} + E_p^{310}x_{Cp} \quad (1)
\]

\[
A_{316} = E_s^{316}x_{Cs} + E_p^{316}x_{Cp} \quad (2)
\]

Substituting the bottom equation into the top, the following relationship is derived:

\[
C_p = \frac{(A_{316}xE_s^{310} - A_{310}xE_s^{316})}{(E_s^{310}xE_p^{316} - E_p^{316}xE_s^{316})} \quad (3)
\]
with the variables defined as follows:

\[ A_{310} \] = absorbance at 310 nm;

\[ A_{316} \] = absorbance at 316 nm;

\[ C_s \] = concentration of p-benzoquinol ether imine and quinol imine.

\[ C_p \] = concentration of product

\[ E_{310}^g \ and \ 316 \] = absorptivity coefficient of imine at 310 and 316 nm

\[ E_{310}^p \ and \ 316 \] = absorptivity coefficient of product at 310 and 316 nm

### Table 9.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Run</th>
<th>Extinction Coefficient (LMol⁻¹cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>310 nm</td>
</tr>
<tr>
<td>41a</td>
<td>1</td>
<td>2121</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2122</td>
</tr>
<tr>
<td></td>
<td>Ave.</td>
<td>2122</td>
</tr>
<tr>
<td>41b</td>
<td>1</td>
<td>2335</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2364</td>
</tr>
<tr>
<td></td>
<td>Ave.</td>
<td>2350</td>
</tr>
<tr>
<td>41c</td>
<td>1</td>
<td>2213</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2242</td>
</tr>
<tr>
<td></td>
<td>Ave.</td>
<td>2228</td>
</tr>
</tbody>
</table>
The reactions were followed at 310 nm and 316 nm since this region contained the most favorable difference in absorptivity of the rearrangement product versus starting p-benzoquinol imine. Two separate runs were done for compounds 41a, 41b, 41c and 41f to calculate the characteristic absorptivity coefficients which are recorded in Table 9 above.

**Scheme XXIV.**
Furthermore preliminary investigations discovered that imine hydrolysis in aqueous acidic media was competitive with phenyl migration. Therefore it became necessary to run the kinetic experiments under dry conditions to ensure accurate rate data. In addition numerous trial runs determined that a $1.4 \times 10^{-4}$ M $\text{CF}_3\text{CO}_2\text{H}$ solution led to a convenient rate of rearrangement in both systems and gave a quantitative yield of the rearrangement products 41c and 41f (Scheme XXIV). The structure of 41f was confirmed via a straightforward methylation of 41c.

Two separate runs were performed with each sample at 25 °C and a summary of the first order rate constants for phenyl migration in $N$-benzoylamino-4-methoxy-4-phenyl-$p$-benzoquinone imine 41a and

<table>
<thead>
<tr>
<th>Compound</th>
<th>Run</th>
<th>Rate Constant $k_{1\text{st order}}$(min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41a</td>
<td>1</td>
<td>$0.46 \times 10^{-2}$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$0.50 \times 10^{-2}$</td>
</tr>
<tr>
<td>41b</td>
<td>1</td>
<td>$0.31 \times 10^{-1}$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$0.35 \times 10^{-1}$</td>
</tr>
</tbody>
</table>
N-benzoylamino-4-hydroxy-4-phenyl-p-benzoquinone imine 41b recorded in Table 10. From the kinetic data, the rate constant for the acid-catalyzed phenyl migration of the p-benzoquinol ether imine 41a was calculated as $0.48 \times 10^{-2} \text{ min}^{-1}$ and $0.33 \times 10^{-1} \text{ min}^{-1}$ for 41b. Thus the relative stabilities of 41a and 41b differ by a relative rate factor of 7 in mild acidic media, a phenomenon undoubtedly due to the better electron-donating ability of the hydroxyl group to facilitate phenyl migration. More importantly, the above kinetic study emphasizes the problems associated with isolation of N-acylated p-benzoquinol imines with good migrating groups such as aryl- and branched alkyl moieties.

Upon completion of the above kinetic study, the hydrolytic behavior of N-acylated p-benzoquinol ether imines was examined as a function of pH. Initial investigations began with the acidic hydrolysis of 44a in acetonitrile with 1.0 N HCl at room temperature. After 1.5 h, work-up and isolation, the dienone 44c was isolated in a 72% yield (Scheme XXV). Interestingly, with basic conditions, a very different outcome was observed. Surprisingly, addition of a 1.0 N NaOH solution to 42a in THF gave a reaction mixture which appeared to consist of the deacylated imine 42d as the major product with minor amounts of the dienone 42c (Scheme XXVI). However, attempts to isolate 42d resulted in complete decomposition of the product. Therefore upon completion of the basic hydrolysis, the reaction mixture was placed under hydrogen with a 5% Pt/C catalyst for 12 h at room temperature, forming 4-aminobiphenyl 42e and p-phenylphenol 42f in 90% and 10%
yield, respectively. Thus assuming the reductive aromatization is quantitative in yield, the deacylated imine 42d is apparently formed in 90% yield with the alkaline hydrolysis (1.0 N NaOH solution) of 42a.

Interestingly 44a is remarkably stable in neutral media. When the hydrolysis of a 0.03 M solution of 44a was attempted with a NaOH/NaH$_2$PO$_4$ buffer solution (pH 7.4), formation of 44c was not observed after 24 h at room temperature. For complete hydrolysis, heating the solution at 55 °C for 20 h gave an 81% yield of 44c. The stability of 44a to neutral media at elevated temperatures is an important observation if in fact the biological intermediate 22 has similar stability. In addition it suggests the problems associated with the hydrolysis of the N-acetyl-p-benzoquinol ether imines during isolation (see Results in Chapter 2) is in fact due to chromatography and not adventitious water during aqueous work-up. To further demonstrate the stability of N-acetyl-p-benzoquinol ether imines to neutral aqueous media, the p-benzoquinol ether imine 42a was dissolved in acetonitrile, saturated NaCO$_3$ solution added and the reaction mixture stirred at room temperature (Scheme XXVI). Interestingly,
complete hydrolysis was observed after 10 days giving a 92\% yield of 4-methoxy-4-phenyl-2,5-cyclohexadienone 42c.

\section*{Scheme XXVI.}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\chem{(O)\text{Ph OCH}_3\text{Ph}}};
\node at (1.5,0) {\chem{(O)\text{N-CPh\text{Ph OCH}_3}}};
\node at (3,0) {1.0 N \text{\text{NaOH}}};
\node at (4.5,0) {\chem{(O)\text{Ph OCH}_3\text{Ph}}};
\node at (6,0) {\chem{(NH)\text{Ph OCH}_3\text{Ph}}};
\node at (0,-2) {42c};
\node at (1.5,-2) {42a};
\node at (3,-2) {42c};
\node at (4.5,-2) {42d};
\node at (0,-4) {42e (90\%)};
\node at (1.5,-4) {42f (10\%)};
\node at (6,-4) {24};
\draw[->] (0,-1) -- (1.5,-1);
\draw[->] (1.5,-1) -- (3,-1);
\draw[->] (3,-1) -- (4.5,-1);
\draw[->] (4.5,-1) -- (6,-1);
\node at (0,-1) {sat. \text{\text{NaHCO}_3}};
\node at (0,-2) {10 days, 92\%};
\node at (1.5,-2) {\text{\text{CH}_3\text{CN, RT}}};
\node at (3,-2) {\text{\text{THF}}};
\end{tikzpicture}
\end{center}

In addition, it should be noted that the hydrolysis of 44a with acidic, alkaline and neutral media detailed above, did not produce the 1,4-addition adduct, e.g., 2-(\text{\text{N-acetylamino})-4-hydroxyfluorene 24.}^{64}

As described in Chapter 1, Novak reported 24 was obtained as a major product from the hydrolytic decomposition of \text{\text{N-(sulfonatoxy)-2-(acetylamino)-fluorene}} in acidic and neutral media.\text{^{40}} In addition Scribner reported that 24 was a major decomposition product of \text{\text{N-acetoxy-N-acetyl-2-aminofluorene}} in a 0.01 M phosphate buffer
solution (pH 7.4) after 2 h at 37 °C (Scheme XXVII). Furthermore he proposed the product was formed from the initially generated p-benzoquinol imine 22, followed by decomposition to 22a, and subsequent lose of water to give 24. Gassman later published work which supported Scribner's mechanistic hypothesis (Scheme XXVIII). He reported that N-acetyl-4-methyl-p-benzoquinol methyl ether imine 37a in acidic methanol generated an equilibrated mixture of 37a, 37c and 37d prior to conversion of 37d to the 1,4-addition adduct 37e in quantitative yield.

Scheme XXVII.

Therefore the hydrolytic behavior of the N-benzoyl-p-benzoquinol ether and quinol imines 45a and 45b were investigated in aqueous acidic media as a model system to examine the importance of the hydroxyl center in p-benzoquinol imines for the formation of Michael addition adducts such as 24 (Scheme XXIX). Interestingly the
addition of trace amounts of 1.0 N HCl solution to 45a and 45b in CH$_3$CN gave the rearrangement products 45c and 45d for the hydrolysis of 45a in 45% and 20% yields respectively, and a 60% yield of 45d for the hydrolysis of 45b, in addition to small amounts of the dienones 45e (15%) and 45f (12%). Surprisingly the 1,4-addition product, e.g., 6-(N-benzoylamino)-4-hydroxyindane was not isolated in either hydrolysis reaction.

The rationalization for the formation of the 1-indanol analogue 45c and the methyl ether derivative 45d may be viewed as initial generation of the quinone methide intermediate l-45b followed by recapture of H$_2$O or CH$_3$OH (Scheme XXX). However the reaction must entail a tight transition state since only partial incorporation of H$_2$O was observed in the hydrolysis of 45a.
In addition to investigating the decomposition products of $N$-acylated p-benzoquinol ether and quinol imines in aqueous media, additional efforts were directed at studying the reactions of these systems with aryl and alkyl amines. Generally the basic amino groups on proteins and peptides are largely protonated under physiological conditions, and therefore would exhibit relatively low nucleophilicity. However, as illustrated in Scheme XXXI, the addition

**Scheme XXIX.**

![Chemical Structures]  

of free alkyl amino groups such as benzyl amine to a THF solution of $N$-benzoyl- and $N$-acetyl-4-phenyl-p-benzoquinol methyl ether imine 41a and 42a at room temperature generates the 1,2-addition adduct
characterized as N-benzyl-4-phenyl-p-benzoquinol methyl ether imine 52 in 82% and 75% yields, respectively. The ¹H and ¹³C NMR spectra of Scheme XXX.

52 are particularly informative: ¹H NMR δ 7.5 - 7.2 (m, 10 H), 6.9 (dd, J = 10, 2 Hz, 1 H), 6.6 (dd, J = 10, 2 Hz, 1 H), 6.3 (dd, J = 10, 2 Hz, 1 H), 6.2 (dd, J = 10, 2 Hz, 1 H), 4.9 (s, 2 H), 3.4 (s, 3 H); ¹³C NMR δ 157, 143, 142, 140, 139, 133, 128, 127, 127, 126, 125, 119,

Scheme XXXI.

52 (75%-82%)
Figure 44. 200 MHz $^1$H NMR Spectrum of 52
Figure 45. 50 MHz $^{13}$C NMR Spectrum of 52
In addition a reductive aromatization of 52 with BH$_3$-THF complex in THF formed N-benzyl-4-aminobiphenyl 53 in 70% yield which corresponded with the melting point and spectral properties of authentic sample (Scheme XXXII). Interestingly, deacylation of the N-acetyl-p-benzoquinol ether imine 42a with 1.0 N NaOH solution followed by the addition of benzyl amine also generates the N-benzyl-p-benzoquinol ether imine 52 in 65% yield (Scheme XXXI). The lower yield is invariably due to an approximate 10% loss of 42a via imine hydrolysis forming 4-methoxy-4-phenyl-2,5-cyclohexadienone 42c.

**Scheme XXXII.**

![Scheme XXXII](image)

In addition to the interesting biological implications of the imine exchange reaction illustrated in Scheme XXXI, this reaction generates a new class of p-benzoquinol ether imines unavailable via
Therefore a number of reactions were performed with different \( N \)-acylated-\( p \)-benzoquinol ether imines and alkyl and aryl amines to emphasize the facility of this imine exchange reaction (Table 11 below). In addition, the reduction of 61 with LiAlH\(_4\) in THF gave a racemic mixture of \( N \)-(p-methoxyphenyl)-\( \alpha \)-methylbenzyl amine 62 in 60% yield. Apparently with these conditions, hydride anion is sufficiently basic to deprotonate at the benzyl position of 61 forming the imine isomer 61a, which upon reduction, results in the total loss of chirality (Scheme XXXIII).

In addition to the above investigations, some collaborative work was undertaken with the research group of Professor Michael Novak at

| Table 11. \( N \)-Alkyl- or \( N \)-Aryl-\( p \)-Benzoquinone Imines. |
|---|---|---|
| **Entry** | **Product** | **Yield (%)** |
| 1 | \[
\begin{array}{c}
\text{Ph} \\
\text{Ph OCH}_3 \\
\end{array}
\] | 45 |
| 2 | \[
\begin{array}{c}
\text{Bu} \\
\text{Ph OCH}_3 \\
\end{array}
\] | 69 |
Table 11. (continued).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>![Image of product 3]</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>![Image of product 4]</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>![Image of product 5]</td>
<td>58(^a)</td>
</tr>
<tr>
<td>6</td>
<td>![Image of product 6]</td>
<td>55(^b)</td>
</tr>
</tbody>
</table>

\(^a\) 60 was isolated as a 1:1 ratio of syn : anti isomers.
\(^b\) The optical rotation of 61 is \([\alpha]_D = +165\)
\(^c\) The above imine exchange reactions were performed via deacylation of the respective N-acetyl-p-benzoquinol ether imine followed by the addition of the amine except for entry 6 which was prepared from N-benzylo1-p-benzoquinone imine dimethyl ketal 30\(^a\) (see Experimental Section for Details).
Miami University of Ohio. Professor Novak has published numerous kinetic investigations on the decomposition reactions of \(N\)-(sulfonatoxy)-\(N\)-acylated-arylamines and has been the central authority in this area. In addition his investigations which described the \(N\)-acylated-\(p\)-benzooquinol imines as important intermediates in the hydrolytic decompositions of \(N\)-(sulfonatoxy)-\(N\)-acylated-arylamines provided much of the emphasis for undertaking the work described in this thesis.

However, as mentioned earlier, \(N\)-acylated-\(p\)-benzoquinol imines have not been available for direct study prior to this investigation. Therefore kinetic investigations were performed on the hydrolytic decomposition of the \(p\)-benzoquinol ether and quinol imines 41a and 41b and comparatively analyzed with the decomposition of \(N\)-(sulfonato-
oxy)-N-acetyl-4-aminobiphenyl 63. Although the investigation is currently in its preliminary stages, initial findings support the contention proposed in this thesis (see Results in Chapter 2) that the N-acetyl-p-benzoquinol imine has a much greater susceptibility for imine hydrolysis relative to the N-benzoyl imine counterpart (Scheme XXXIV). For example the kinetic rate data for the

hydrolytic decomposition of 63 document a 50 fold increase in the rate of imine hydrolysis for 51a ($k_1$) relative to the rate of imine
hydrolysis for 41b (k_2). In addition the rate of imine hydrolysis for 41a (k_3) has been calculated to be 2 - 3 times slower relative to k_2.

In summary, the reactivity of 4-substituted p-benzoquinol imines have been investigated in aqueous acidic, neutral, and basic media as well as with nucleophilic species such as aryl and alkyl amines. Interestingly the N-acetyl-p-benzoquinol imines such as 44a are very stable to neutral aqueous media, but hydrolyze readily in acidic media. However with highly alkaline solution, deacylation of the N-acetyl-imine linkage is observed in high yield. Finally, addition of alkyl and aryl amines to N-acylated p-benzoquinol ether imines form 1,2-addition adducts, e.g., N-alkyl- and N-aryl-p-benzoquinol ether imines in 65% - 80% yield, which are not available via traditional electrochemical methodology.
Experimental

General Procedure

Melting points were determined in capillaries in a Thomas-Hoover "Unimelt" apparatus and are uncorrected. IR spectra were determined on a Perkin-Elmer Model 283B spectrometer. Unless noted otherwise, the $^1$H NMR spectra were measured on a Bruker 200 MHz $^1$H NMR using deutereochloroform as solvent and residual chloroform as standard. Mass spectral and exact mass measurements were obtained by Mr. Richard Weisenberger on a Kratos MS-30 spectrometer connected to a DS-55 data system. Combustion analyses were performed by Scandinavian Microanalytical Laboratory, Herlev, Denmark, and M-H-W Laboratories, Phoenix, Arizona. All reagents or compounds not explicitly referenced were obtained from the Aldrich Chemical Co. Alumina and silica gel (Kieselgel 60 230-400 mesh) were obtained from E. Merck Co and Florisil (100 - 200 mesh) purchased from Fischer Scientific Co. Thin-layer chromatography (TLC) was done using Merck silica gel 60 F$_{254}$ pre-coated aluminum backed plates, 0.2-mm thickness. Visualization was by UV or by spraying with 5% ethanolic phosphomolybdic acid and then heating. Tetrahydrofuran was purified by distillation from benzophenone ketyl. Throughout the Experimental Section the following abbreviations are used: petroleum ether, bp 35-60 °C (PE), hexanes, bp 68-69 °C (H), ethyl acetate (EtOAc), tetrahydrofuran (THF), $p$-toluene sulfonic acid ($p$-TsOH), and thin layer chromatography (TLC).
Extractive workup refers to extraction of the material into the indicated solvent, washing the organic layer with brine solution, drying over Drierite ($\text{CaSO}_4$), concentration in vacuo, and drying to constant weight under vacuum (1-2 Torr).

**Kinetic Measurements.** All kinetics were performed in dry $\text{CH}_2\text{Cl}_2$ with $1.4 \times 10^{-4}$ M $\text{CF}_3\text{CO}_2\text{H}$ solution at 25.0 °C. Dry $\text{CH}_2\text{Cl}_2$ was obtained via distillation of reagent grade $\text{CH}_2\text{Cl}_2$ over CaH$_2$ and stored under nitrogen. Kinetic data was gathered by monitoring the changes in UV absorbance at 310 nm and 316 nm of solutions containing either the $N$-benzoyl-4-hydroxy- or $N$-benzoyl-4-methoxy-4-phenyl-p-benzoquinol imine. A Beckman Model DU-7 uv-vis spectrometer equipped with thermostated cell holders was used to record absorbance changes. Wavelength scans were taken in the range of 300 to 350 nm and the change in absorbance at the two wavelengths recorded. Kinetic measurements were taken every 3.0 min for the 4-hydroxy-p-benzoquinol imine $41b$ and every 10.0 min for the 4-methoxy-p-benzoquinol imine $41a$ and followed up to 2 half-lives.

A stock solution for $41a$ ca. $3.0 \times 10^{-4}$ M and $41b$ ca. $2.2 \times 10^{-4}$ M was prepared by measuring the weight of p-benzoquinol imine in a volumetric flask using a Mettler AJ100 analytical balance, adding dry $\text{CH}_2\text{Cl}_2$ and placing under nitrogen. To assure a constant concentration of acid, a new stock solution of $8.5 \times 10^{-3}$ M $\text{CF}_3\text{CO}_2\text{H}$ was prepared prior to each kinetic measurement. A 10 mL volumetric flask was evacuated with nitrogen and 10.0 - 10.7 mg of $\text{CF}_3\text{CO}_2\text{H}$ added with
syringe. The exact weight of dry CH$_2$Cl$_2$ to prepare an 8.5 x 10$^{-3}$ M solution was then calculated and added with syringe and the solution kept under nitrogen prior to use. Each kinetic run was prepared by the addition of 3.0 mL of the p-benzoquinol imine stock solution with graduated pipet to a dry UV cell followed by the addition of 50 microliters of 8.5 x 10$^{-3}$ M CF$_3$CO$_2$H solution with a pre-calibrated 250 microliter syringe to give an acid solution concentration of 1.4 x 10$^{-4}$ M. The UV cell was capped with a glass stopper, shaken vigorously for approximately 10 seconds, and placed in the thermostated UV cell holder for the remainder of the kinetic run. The 250 microliter syringe was calibrated with degassed 'Baker analyzed' reagent grade water.

**Product Analyses.** Product studies were performed under the same conditions as those employed in the kinetic studies, however higher imine concentrations for N-benzoylamino-4-methoxy-4-phenyl-benzoquinol imine 41a and N-benzoylamino-4-hydroxy-4-phenyl-benzoquinol imine 41b were used to facilitate isolation and characterization. The purity of the products was monitored by comparison of the $^1$H NMR spectra and melting points with authentic samples. For both imine isomers, a quantitative yield of the phenyl-migrated product was obtained. The experimental procedures are described as follows:
Addition of CF$_3$CO$_2$H to 41a

*N*-Benzoyl-4-methoxy-4-phenyl-p-benzoquinol imine 41a (30 mg, 0.1 mmol) was placed in a dry 25 mL round-bottom flask and dissolved in dry CH$_2$Cl$_2$ (3.0 mL) under nitrogen. A solution of 8.5 x 10$^{-3}$ M CF$_3$CO$_2$H (50 µl) was then added, the flask stoppered, and the reaction mixture stirred at room temperature for 24 h. The solvent was then removed in vacuo, and the product placed under vacuum (0.2 torr) for 5 h giving a white solid (30 mg, 100% yield) which was characterized as 41f with absolute purity by $^1$H NMR (200 MHz). The melting point and spectral properties corresponded to authentic sample (see Preparation of 41f below).

Addition of CF$_3$CO$_2$H to 41b

*N*-Benzoyl-4-hydroxy-4-phenyl-p-benzoquinol imine 41b (10 mg, 0.035 mmol) was placed in a dry 25 mL round-bottom flask and dissolved in dry CH$_2$Cl$_2$ (3.0 mL) under nitrogen. A solution of 8.5 x 10$^{-3}$ M CF$_3$CO$_2$H (50 µl) was then added, the flask stoppered, and the reaction mixture stirred at room temperature for 10 h. The solvent was then removed in vacuo, and the product dried under vacuum (0.2 torr) for 2 h giving a white solid (10 mg, 100% yield) which was characterized as 41c with absolute purity by $^1$H NMR (200 MHz). The melting point and spectral properties corresponded to authentic sample (see Preparation of 41c in Experimental in Chapter 2).
Preparation of 41f

The biphenol 41c (100 mg, 0.3 mmol) and anhydrous K$_2$CO$_3$ (500 mg, 3.0 mmol) were placed in a dry 25 mL round-bottom flask and evacuated with nitrogen. Dry THF (4.0 mL) was added followed by the addition of excess CH$_3$I (1 mL). The reaction mixture was stirred vigorously at room temperature, monitored by TLC (20% EtOAc/H used as eluant) and complete after 23 h. Filtration to remove K$_2$CO$_3$, combination of the filtrates and removal of solvent in vacuo gave the product as a white solid (110 mg, 95 % yield), mp 189.0 - 190.0 °C. IR (KBr) 3250, 1640, 1520, 1480, 1400, 1225 cm$^{-1}$; $^1$H NMR $\delta$ 7.9 (br s, 1 H), 7.8 (d, 2 H), 7.6 (dd, J = 8, 1.5 Hz, 1 H), 7.5 - 7.3 (m, 9 H); 6.9 (d, J = 8 Hz, 1 H), 3.8 (s, 3 H); HRMS Calcd for C$_{20}$H$_{17}$O$_2$: m/e 303.1255, obsd 303.1272.

Hydrolysis of 44a with NaOH/NaH$_2$PO$_4$ Buffer Solution, pH 7.4

The p-benzoquinol imine 44a (68 mg, 0.27 mmol) was dissolved in CH$_3$CN (5 mL), a 1.0 N NaOH/1.0 M NaH$_2$PO$_4$ buffer solution (pH 7.4) added and the homogenous reaction mixture heated in an oil bath to 55 °C. The hydrolysis was monitored by TLC (40% EtOAc/H) every 5.0 h and complete after 20
h. Extractive workup with CH$_2$Cl$_2$ (2 x 15 mL) gave a brown solid (50 mg) as crude product. Column chromatography was performed with a Florisil support (10 cm x 2 cm column) and 20% EtOAc/H used as eluant. The product 44c ($R_f$ = 0.56, 40% EtOAc/H) was isolated as a light yellow solid (46 mg, 81% yield), mp 101.0 - 102.0 °C. A recrystallization from CH$_2$Cl$_2$/Et$_2$O/H at 0 °C gave a white solid, mp 102.0 - 103.5 °C. IR (KBr) 1669, 1640, 1608, 1473, 1462, 1387, 1282, 1088, 1076, 1051, 949, 925, 810, 762, 716 cm$^{-1}$; $^1$H NMR δ 7.5 - 7.3 (m, 4 H), 7.2 (d, $J$ = 10 Hz, 1 H), 6.47 (dd, $J$ = 10, 2 Hz, 1 H), 6.3 (d, 2 Hz, 1 H), 4.05 (dd, $J$ = 20, 1.5 Hz, 1 H), 3.3 (d, $J$ = 20 Hz, 1 H), 3.2 (s, 3 H); HRMS Cald for C$_{13}$H$_{12}$O: m/e 181.0651, obsd 181.0647.

**Hydrolysis of 44a with 1.0 N HCl Solution**

The p-benzoquinol imine 44a (62 mg, 0.25 mmol) was placed in a 25 mL round-bottom flask, CH$_3$CN (5 mL) added, and the yellow solution equipped with a stir bar. A 1.0 N HCl solution (0.5 mL) was added and the reaction mixture stirred vigorously at room temperature. The reaction was monitored by TLC (40% EtOAc/H) and appeared complete after 2.0 h and dark brown in color. Saturated NaHCO$_3$ solution (10 mL) was added and the mixture stirred vigorously to insure complete neutralization. Extractive workup was performed with CH$_2$Cl$_2$ (2 x 20 mL) giving a brown oily solid (50 mg) as crude product. Column chromatography was performed with a Florisil support (10 cm x 2 cm column) and 20% EtOAc/H used as eluant. The product 44c ($R_f$ = 0.56, 40% EtOAc/H) was isolated as a light yellow
solid (38 mg, 72% yield), mp 95.0 - 97.0 °C. All spectral properties corresponded with authentic sample.

**Hydrolysis of 42a with 1.0 N NaOH Solution**

*N*-acetyl-4-phenyl-2-benzoquinol imine 42a (80 mg, 0.33 mmol) was placed in a 25 mL round-bottom flask, charged with a stir bar and dissolved in THF (5 mL). A solution of 1.0 N NaOH was then added and the heterogeneous mixture stirred vigorously at room temperature. After 3.0 h starting material could not be observed by TLC (40% EtOAc/H). The reaction mixture was then extracted with distilled THF (2 x 30 mL), the organic layer dried over Na$_2$SO$_4$ and placed in a Paar hydrogenation bottle. A 10% Pt/C catalyst was then added and the contents placed under hydrogen (65 psi) for 12 h at room temperature. The reaction mixture was filtered through celite, the filtrate dried over CaSO$_4$ and the solvent removed in vacuo giving a light brown oily solid (100 mg). Column chromatography was performed using flash silica gel (10 cm x 1 cm column) and 10% EtOAc/H as eluant. The first component was isolated as a white solid (6 mg, 10% yield) with spectral properties consistent with p-phenylphenol ($R_f$ = 0.31, 40% EtOAc/H), mp 156 - 158 °C (lit. mp 164 - 166 °C). The second component was isolated as a light brown solid (50 mg, 90% yield) and characterized as 4-aminobiphenyl ($R_f$ = 0.41, 40% EtOAc/H), mp 46.0 - 48.0 °C (lit. mp 53 - 55 °C) and spectral properties corresponding with authentic sample.
Hydrolysis of 42a with Saturated NaHCO<sub>3</sub> Solution

The p-benzoquinol imine 42a (90 mg, 0.37 mmol) was placed in a 25 mL round-bottom flask, CH<sub>3</sub>CN (10 mL) added, followed by the addition of saturated NaHCO<sub>3</sub> solution giving a heterogeneous reaction mixture. The reaction was monitored by TLC (40% EtOAc/H) daily and appeared to be complete after 10 days. Addition of H<sub>2</sub>O (5 mL) and extractive workup with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL) gave a yellow oil (80 mg) as crude product. Purification with column chromatography was performed with a Florisil support (10 cm x 1 cm column) using 10% EtOAc/H as eluant. The product 42c (R<sub>f</sub> = 0.86, 40% EtOAc/H) was isolated as a white solid (70 mg, 92% yield), mp 85.0 - 87.0 °C, and the melting point and spectral properties corresponding with authentic sample (see Preparation of 42a in Experimental of Chapter 2).

Hydrolysis of 45a with 1.0 N HCl Solution

The p-benzoquinol imine 45a (100 mg, 0.37 mmol) was placed in a 25 mL round-bottom flask, charged with stir bar, and dissolved in CH<sub>3</sub>CN (5 mL). A 1.0 N HCl solution (1 mL) was added and the solution stirred vigorously at room temperature. The reaction was monitored by TLC (40% EtOAc/H) and complete after 45 minutes. Addition of NaHCO<sub>3</sub> (5 mL) and extractive workup (CH<sub>2</sub>Cl<sub>2</sub>, 2 x 15 mL) gave a yellow oil (85 mg) as the crude
product. Column chromatography was performed with a silica gel-supported column (base-washed with 5% NH₄OH/CH₃OH and dried under vacuum, 25 cm x 2 cm, 230 - 400 mesh) using 25% EtOAc/H (100 mL) and 50% EtOAc/H (200 mL) as eluant. The first component was isolated as a light yellow oil (10 mg, 15% yield) and characterized as 45e (Rf = 0.63, 40% EtOAc/H). The next component was isolated as a clear colorless oil (45 mg, 45% yield) and characterized as 45c (Rf = 0.57, 40% EtOAc/H) followed by the isolation of 45d (Rf = 0.3, 40% EtOAc/H) as a yellow solid (19 mg, 20% yield), mp 112.0 - 114.0 °C. 45c: IR (neat) 1650, 1601, 1580, 1536, 1494, cm⁻¹; ¹H NMR δ 7.9 (dd overlapped by broad singlet, J = 8.0 Hz, 3 H), 7.7 (dd, J = 1.5 Hz, 1 H), 7.6 - 7.4 (m, 4 H), 7.2 (d, J = 8.0 Hz, 1 H), 4.8 (m, 1 H), 3.4 (s, 3 H), 3.0 (m 1 H), 2.7 (m, 1 H), 2.4 (m, 1 H), 2.1 (m, 1 H); HRMS Calcd for C₁₇H₁₇O₂: m/e 267.1255, obsd 267.1228.

**Hydrolysis of 45b with 1.0 N HCl Solution**

The p-benzoquinol imine 45b (70 mg, 0.028 mmol) was placed in a 25 mL round-bottom flask, charged with stir bar, and dissolved in CH₃CN (5 mL). A 1.0 N HCl solution (1 mL) was added and the solution stirred vigorously at room temperature.

The reaction was monitored by TLC (40% EtOAc/H) and complete after 45 minutes. Addition of NaHCO₃ and extractive workup (CH₂Cl₂, 2 x 15 mL) gave a yellow oil (50 mg) as the crude product. Column
chromatography was performed with a silica gel-supported column (base-washed with 5% NH₄OH/CH₃OH and dried under vacuum, 25 cm x 2 cm, 230 - 400 mesh) using 25% EtOAc/H (100 mL) and 50% EtOAc/H (200 mL) as eluant. The dienone 45f was isolated as a clear colorless oil (5 mg, 12% yield) and characterized via ¹H NMR: δ 7.0 (d, J = 10 Hz, 1 H), 6.1 (dd, J = 10.0 Hz, 2.0 Hz, 1 H), 6.0 (d, J = 2.0 Hz, 1 H), 3.0 - 2.0 (m, 6 H). However due to the small amount of material obtained and the instability of 45f (Rf = 0.28, 40% EtOAc/H) further characterization was not attempted. The major component 45d (Rf = 0.20, 40% EtOAc/H) was isolated as a clear colorless oil which upon addition of CH₂Cl₂/Et₂O and scratching with spatula gave a white crystalline solid (42 mg, 60% yield), mp 114.0 - 116.0 °C. Analytically pure material was obtained via a recrystallization from CH₂Cl₂/H, mp 115.0 - 116.0 °C. IR (KBr) 3310, 1650, 1603, 1537, 1493, 707 cm⁻¹; ¹H NMR (D₂O) δ 8.4 (s, 1 H), 7.8 (d, J = 8 Hz, 2 H), 7.6 (s, 1 H), 7.5 - 7.3 (m, 4 H), 7.1 (d, J = 8 Hz, 1 H), 5.1 (t, J = 6 Hz, 1 H), 2.9 (m, 1 H), 2.7 (m, 1 H), 2.4 (m, 1 H), 1.9 (m, 1 H); HRMS Calcd for C₁₆H₁₅O₂: m/e 253.1099, obsd 253.1144.
Preparation of 52 from 41a.

N-Benzoyl-4-methoxy-4-phenyl-p-benzoquinol imine (72 mg, 0.24 mmol) was placed in a 25 ml round-bottom flask, charged with a stir bar and dissolved in THF (5 mL). Benzylamine (0.03 mL, 0.26 mmol) was added and the solution stirred vigorously at room temperature. The reaction was monitored by TLC (20% EtOAc/H) and complete after 2.0 h. The reaction solvent was removed in vacuo giving a yellow oil (100 mg) as crude product. The oil was dissolved in CH₂Cl₂ and impregnated on florisil (1.5 g). Column chromatography was performed with a florisil-packed column (10 cm x 2 cm, column packed with hexane) and 15% EtOAc/H (150 mL) used as eluant. The product came off the column as a light yellow band but the eluant clear and colorless. Removal of solvent in vacuo gave a light yellow oil (56 mg, 82% yield) which was greater than 95% pure by ¹H NMR (200 MHz). IR (neat) 2932, 1602, 1583, 1489, 1449, 1069, 823 cm⁻¹; ¹H NMR δ 7.5 - 7.2 (m, 10 H), 6.9 (dd, J = 10, 2 Hz, 1 H), 6.6 (dd, J = 10, 2 Hz, 1 H), 6.3 (dd, J = 10, 2 Hz, 1 H), 6.2 (dd, J = 10, 2 Hz, 1 H), 4.9 (s, 2 H), 3.4 (s, 3 H); ¹³C NMR δ 157, 143, 142, 140, 139, 133, 128, 128, 127, 127, 126, 125, 119, 77, 55, 52; HRMS calcd for C₂₀H₁₉NO m/e 289.1462 obsd 289.1471.
Preparation of 52 from 42a

*N*-Acetyl-4-methoxy-4-phenyl-p-benzoquinol imine 42a (114 mg, 0.47 mmol) was placed in a 25 ml round-bottom flask, charged with a stir bar and dissolved in THF (5 mL). Benzyamine (0.051 mL, 0.5 mmol) was added and the mixture stirred vigorously at room temperature. The reaction was monitored by TLC (20% EtOAc/H) and complete after 2.0 h. The reaction solvent was removed in vacuo giving a yellow oil (165 mg) as crude product. The oil was dissolved in CH₂Cl₂ and impregnated on Florisil (1.5 g). Column chromatography was performed with a Florisil support (15 cm x 1 cm column, column packed with hexane) and 15% EtOAc/H (150 mL) used as eluant. The product came off the column as a light yellow band but the eluant clear and coloress. Removal of solvent in vacuo gave a light yellow oil (97 mg, 74% yield) which was greater than 95% pure by ¹H NMR (200 MHz).

Preparation of 52 via Deacylation of 42a and Addition of Benzyamine

*N*-Acetyl-4-methoxy-4-phenyl-p-benzoquinol imine 42a (100 mg, 0.41 mmol) was placed in a 25 ml round-bottom flask, charged with a stir bar and dissolved in THF (5 mL). A 1.0 N NaOH (2 mL) solution was then added and the heterogeneous mixture stirred vigorously at room temperature. After 2 h the reaction was determined to be complete by TLC (20% EtOAc/H). The stirring was stopped allowing the nonhomogenous layers to separate and benzy amine (0.045 mL, 0.41 mmol) added with syringe. Vigorous stirring was resumed at
room temperature for 6 h. The reaction mixture was then transferred to a separatory funnel and extracted with ether (3 x 25 mL). The organic layer was separated, dried through Na$_2$SO$_4$ and the solvent removed in vacuo giving a yellow oil (110 mg). The oil was dissolved in CH$_2$Cl$_2$ and impregnated on Florisil (0.8 g). Column chromatography was then performed with a Florisil support (15 cm x 1 cm column, column packed with hexane) and hexane (100 mL) and 5% EtOAc/H (100 mL) used as eluant. The product came off the column as a light yellow band but the eluant clear and colourless. Removal of solvent in vacuo gave a light yellow oil (78 mg, 65% yield) which was greater than 95% pure by $^1$H NMR (200 MHz).

**Preparation of N-Benzyl-4-aminobiphenyl 54**

4-aminobiphenyl (500 mg, 3.0 mmol) was placed in a 50 mL round-bottom flask, dissolved in pyridine (8 mL) and equipped with stir bar and reflux condenser. Benzyl chloride (0.345 mL, 3.0 mmol) was then added and the solution heated to reflux for 48 h. The rxn mixture was then cooled to room temperature, and extracted with CH$_2$Cl$_2$ (4 x 30 mL). The organic layer was then dried through Na$_2$SO$_4$, and the solvent removed in vacuo to give a red-brown solid (783 mg). Column chromatography was performed on flash silica
gel (15 cm x 2 cm column) using 5% EtOAc/H as eluant. The di-benzylated product was the first compound off the column followed by the desired mono-benzylated product. 

N,N-Dibenzyl-4-aminobiphenyl 54 was isolated as a white solid (140 mg, 13% yield), mp 110.0 - 112.0 °C. N-Benzyl-4-aminobiphenyl 55 was isolated as a pale white crystalline solid (452 mg, 59% yield), mp 87.0 - 89.0 °C (lit. mp, 69 100 - 101 °C). 54: IR (KBr) 3399 (w), 1611, 1526, 1488, 1447, 1400, 1202, 823, 758, 694 cm⁻¹; ¹H NMR δ 7.6 - 7.2 (m, 12 H), 6.7 (d, J = 8.5 Hz, 2 H), 4.4 (s, 2 H). 55: IR (KBr) 1613, 1525, 1493, 1451, 1398, 1237 cm⁻¹; ¹H NMR δ 7.6 - 7.2 (m, 19 H), 6.9 (d, J = 9.0 Hz, 2 H), 4.8 (s, 4 H).

Reduction of 52 with BH₃-THF Complex

N-Benzyl-4-methoxy-4-phenyl-p-benzoquinol imine 52 (65 mg, 0.23 mmol) was placed in a dry 50 mL round bottom flask, charged with a stir bar, stoppered and evacuated with nitrogen. Dry THF (4 mL) was then added with syringe followed by the addition of BH₃-THF complex to the bright yellow solution. After 12.5 h at room temperature the reaction mixture became clear and colorless. A 5% NaOH solution (10 mL) was added and extraction performed with ether (2 x 25 mL). The organic layer was dried with Na₂SO₄ and the solvent removed in vacuo to give a white sticky solid (62 mg). The solid was impregnated on flash silica gel (0.5 g) and column chromatography was performed with flash silica gel (10 cm x 1 cm column) using 15% EtOAc/H as eluant. The first compound off the
column was isolated as a light yellow waxy solid (40 mg, 70% yield), mp 88.0 - 89.0 °C. Spectroscopic data, IR and \textsuperscript{1}H NMR, corresponded with \textit{N}-benzyl-4-aminobiphenyl 54.

\textbf{Preparation of 56}

\textit{N}-Acetyl-4-methoxy-4-phenyl-\textit{p}-benzoquinol imine (80 mg, 0.33 mmol) was placed in a 25 ml round-bottom flask, charged with a stir bar and dissolved in THF (10 mL). A 1.0 N NaOH solution (5 mL) was then added and the heterogeneous mixture stirred vigorously at room temperature. After 2.0 h the reaction was determined to be complete by TLC (20% EtOAc/H). The stirring was stopped allowing the nonhomogenous layers to separate, 4-aminobiphenyl (56 mg, 0.33 mmol) added and vigorous stirring continued at room temperature. After 3.0 h freshly ground NaBH\textsubscript{4} (200 mg, 5.3 mmol) was added and the reaction run at room temperature for 24 h. The reaction mixture was then transferred to a separatory funnel and extracted with ether (3 x 25 mL). The organic layer was separated, dried through Na\textsubscript{2}SO\textsubscript{4} and the solvent removed in vacuo to give a bright yellow oil (140 mg). The oil was dissolved in CH\textsubscript{2}Cl\textsubscript{2} and impregnated on 5% NH\textsubscript{4}OH/MeOH washed flash silica gel (0.3 g). Column chromatography was then performed with base-washed flash silica gel (washed with 5% NH\textsubscript{4}OH/CH\textsubscript{3}OH, then dried under vacuum, 10 cm x 1 cm column, column packed with hexane) and
25% EtOAc/H used as eluant. The first compound off the column was isolated as a bright yellow oil (52 mg, 45% yield) and characterized as 56 which was greater than 95% pure by \( ^1H \) NMR (200 MHz). IR (neat) 2932, 1599, 1581, 1448, 1221, 1174, 1070, 1008, 851, 825, 757, 696 cm\(^{-1} \); \( ^1H \) NMR \( \delta \) 7.6 - 7.5 (m, 4 H), 7.5 - 7.2 (m, 8 H), 6.9 (d, J = 8 Hz, 2 H), 6.76 (dd, J = 10, 2 Hz, 1 H), 6.6 (dd, J 10, 2 Hz, 1 H), 6.43 (dd, J = 10, 2 Hz, 1 H), 6.33 (dd, J = 10, 2 Hz, 1 H), 3.4 (s, 3 H); \( ^{13}C \) NMR \( \delta \) 156, 144, 142, 141, 131, 128 128, 127, 127, 126, 126, 125, 121, 52; HRMS Calcd for C\(_{25}\)H\(_{21}\)ON m/e 351.1618, obsd 351.1627.

**Preparation of 57**

\( N \)-Acetyl-4-methoxy-4-phenyl-p-benzoquinol imine (115 mg, 0.48 mmol) was placed in a 25 ml round-bottom flask, charged with a stir bar and dissolved in THF (10 mL). A 1.0 N NaOH solution (5 mL) was then added and the heterogeneous mixture stirred vigorously at room temperature. After 2.0 h the reaction was determined to be complete by TLC (20% EtOAc/H). The stirring was stopped allowing the nonhomogenous layers to separate and 4-tert-butylaniline (0.076 mL, 0.48 mmol) added with syringe. Vigorous stirring was resumed at room temperature for 1.0 h and found to be complete by TLC (20% EtOAc/H). The reaction mixture was then transferred to a separatory funnel and extracted with ether (3
x 25 mL). The organic layer was separated, dried through Na$_2$SO$_4$ and the solvent removed in vacuo to give a bright yellow oil (160 mg). The oil was dissolved in CH$_2$Cl$_2$ and impregnated on Florisil (0.5 g). Column chromatography was then performed with a Florisil support (10 cm x 1 cm column, column packed with hexane) and 5% EtOAc/H used as eluant. The product came off the column as a bright yellow band. Removal of solvent in vacuo gave a bright yellow oil (100 mg, 69% yield) which was greater than 95% pure by $^1$H NMR (200 MHz). IR (neat) 2961, 2902, 1601, 1582, 1499, 1488, 1448, 1172, 1086, 1071, 1010 cm$^{-1}$; $^1$H NMR $\delta$ 7.5 - 7.2 (m, 7 H), 6.82 (d, J = 8 Hz, 2 H), 6.73 (dd, J = 10, 2 Hz, 1 H), 6.6 (dd, J = 10, 2 Hz, 1 H), 6.4 (dd, J = 10, 2 Hz, 1 H), 6.27 (dd, J = 10, 2 Hz, 1 H), 3.4 (s, 3 H), 1.3 (s, 9 H); $^{13}$C NMR $\delta$ 157, 147, 143, 142, 141, 132, 129, 128, 126, 121, 120, 77, 57, 34, 32; HRMS Calcd for C$_{23}$H$_{25}$ON m/e 331.1930, obsd 331.1967.

Preparation of 58

$N$-Acetyl-4-methoxy-4-phenyl-p-benzoquinolimine (210 mg, 0.87 mmol) was placed in a 25 ml round-bottom flask, charged with a stir bar and dissolved in THF (10 mL). A 1.0 N NaOH (5 mL) solution was then added and the heterogeneous mixture stirred vigorously at room temperature. After 2.5 h the reaction was determined to be complete by TLC (75% EtOAc/H). The stirring was
stopped allowing the nonhomogenous layers to separate and 3,4-dimethoxyphenethyl amine (0.15 mL, 0.87 mmol) added with syringe. Vigorous stirring was resumed at room temperature for 50 minutes and found to be complete by TLC (75% EtOAc/H). The reaction mixture was then transferred to a separatory funnel and extracted with ether (3 x 25 mL). The organic layer was separated, dried through Na$_2$SO$_4$ and the solvent removed in vacuo to give a bright yellow oil (355 mg). The oil was dissolved in CH$_2$Cl$_2$ and impregnated on Florisil (1.0 g). Column chromatography was then performed with a Florisil support (15 cm x 1 cm column, column packed with hexane) and 40% EtOAc/H used as eluant. The product came off the column as a yellow band but the eluant clear and colorless. Removal of solvent in vacuo gave a clear colorless oil (260 mg, 82% yield) which was greater than 95% pure by $^1$H NMR (200 MHz). IR (neat) 2934, 1605, 1587, 1516, 1489, 1464, 1449, 1262, 1237, 1156, 1140, 1087, 1071, 1030, 823, 755 cm$^{-1}$; $^1$H NMR $\delta$ 7.4 - 7.2 (m, 5 H), 6.77 (s, 2 H), 6.75 (s, 1 H), 6.59 (AB q, J = 12, 10 Hz, 2 H), 6.2 (d, J = 10 Hz, 2 H), 3.88 (t, J = 7 Hz, 2 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 3.3 (s, 3 H), 3.0 (t, J = 7 Hz, 2 H); $^{13}$C NMR $\delta$ 157, 149, 148, 142, 141, 139, 132 (2C), 128, 127, 126, 121, 119, 112, 111, 77, 56 (2C), 53, 52, 37; HRMS Calcd for C$_{23}$H$_{25}$O$_3$N m/e 363.1828, obsd 363.1833.
Preparation of 59

N-Acetyl-p-benzoquinone imine dimethyl ketal (105 mg, 0.54 mmol) was placed in a 25 ml round-bottom flask, charged with a stir bar and dissolved in THF (10 mL). A 1.0 N NaOH solution (5 mL) was then added and the heterogeneous mixture stirred vigorously at room temperature. After 20 minutes the reaction was determined to be complete by TLC (40% EtOAc/H). The stirring was stopped allowing the nonhomogenous layers to separate and 3,4-dimethoxyphenethyl amine (0.09 mL, 0.54 mmol) added with syringe. Vigorous stirring was resumed at room temperature for 1.0 h. The reaction mixture was then transferred to a separatory funnel and extracted with ether (3 x 25 mL). The organic layer was separated, dried through Na₂SO₄ and the solvent removed in vacuo to give a light yellow oil (180 mg). The oil was dissolved in CH₂Cl₂ and impregnated on Florisil (0.5 g). Column chromatography was then performed with a Florisil support (10 cm x 1 cm column, column packed with hexane), Et₃N (5 mL) flushed through the column prior to addition of material, and 65% EtOAc/H used as eluant. The first compound off the column was isolated as a clear light yellow oil (110 mg, 64% yield) and characterized as 59 which was greater than 95% pure by ¹H NMR (200 MHz). IR (neat) 2938, 1588, 1514, 1461, 1262, 1236, 1142, 1106, 1061, 1031, 958 cm⁻¹; ¹H NMR δ 6.7 - 6.6 (m, 3 H), 6.57 (dd, J = 10, 2 Hz, 1 H), 6.44 (dd, J = 10, 2 Hz, 1 H),
6.24 (d, J = 10 Hz, 2 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.80 (t, buried under singlet peaks, 2 H), 3.2 (s, 6 H), 2.9 (t, J = 7 Hz, 2 H); $^{13}$C NMR δ 156, 149, 148, 136, 134, 133, 120, 119, 112, 111, 94, 55, 55, 53, 50, 37; HRMS Calcd for C$_{18}$H$_{23}$O$_4$N m/e 317.1621, obsd 317.1613.

**Preparation of 60**

$N$-Acetyl-3-methoxy-$p$-benzoquinone imine dimethyl ketal (100 mg, 0.44 mmol) was placed in a 25 ml round-bottom flask, charged with a stir bar and dissolved in THF (2 mL). A 1.0 N NaOH solution (1 mL) was then added and the heterogeneous mixture stirred vigorously at room temperature. After 0.5 h the reaction was determined to be complete by TLC (10% MeOH/CHCl$_3$). The stirring was stopped allowing the nonhomogeneous layers to separate and 3,4-dimethoxyphenethyl amine (0.075 mL, 0.44 mmol) added with syringe. Vigorous stirring was resumed at room temperature for 1.0 h. The reaction mixture was then transferred to a separatory funnel and extracted with ether (3 x 25 mL). The organic layer was separated, dried through Na$_2$SO$_4$ and the solvent removed in vacuo to give a light yellow oil (185 mg). The oil was dissolved in CH$_2$Cl$_2$ and impregnated on Florisil (0.5 g). Column chromatography was then performed with a Florisil support (10 cm x 1 cm column, column packed with 50% EtOAc/H) and 50% EtOAc/H (100 mL) and EtOAc (200 mL)
used as eluant. The product came off the column as a light yellow band. Removal of solvent in vacuo gave a light yellow oil (90 mg, 58% yield) which was a mixture of syn/anti- isomers by $^1$H NMR (200 MHz). IR (neat) 2937, 1609, 1587, 1514, 1461, 1263, 1239, 1140, 1081, 1030, 736 cm$^{-1}$; $^1$H NMR $\delta$ 7.2 - 6.7 (m, 6 H), 6.62 (dd, J = 10, 1.5 Hz, 1 H), 6.47 (dd, J = 10, 1.5 Hz, 1 H), 6.1 (d, J = 10 Hz, 1 H), 6.0 (d, J = 10 Hz, 1 H), 5.76 (d, J = 1.5 Hz, 1 H), 5.66 (d, J = 1.5 Hz, 1 H), 3.80 (s, 6 H), 3.79 (s, 6 H), 3.7 (t, J = 7 Hz, 4 H), 3.6 (s, 6 H), 3.2 (s, 12 H), 2.95 (t, J = 7 Hz, 4 H); $^{13}$C NMR $\delta$ 162, 159, 158, 149, 148, 135, 135, 133, 133, 131, 121, 121, 120, 112, 112, 111, 106, 96, 93, 56, 55, 55, 54, 53, 51, 37, 37; HRMS Calcd for C$_{19}$H$_{25}$O$_3$N m/z 347.1726, obsd 347.1731.

**Preparation of 61**

$N$-Benzoyl-$p$-benzoquinone imine dimethyl ketal (135 mg, 0.70 mmol) was dissolved in THF (5 mL), R-$\alpha$-methylbenzylamine (0.09 mL, 0.70 mmol, $[\alpha]_\text{Na}^\circ +38$) added and the solution stirred vigorously at room temperature. The reaction was monitored by TLC (40% EtOAc/H) and complete after 10 h. Extractive workup with CH$_2$Cl$_2$ (3 x 25 mL) gave a yellow oil (272 mg) as crude product. The oil was impregnated on Florisil (2.0 g) and column chromatography performed with a Florisil support (10 cm x 2 cm column) using 20% EtOAc/H as eluant. The product 61 ($R_f = 0.52$,
40% EtOAc/H) was isolated as a light yellow oil (105 mg, 58% yield) which was greater than 95% pure by $^1$H NMR. $[\alpha]_D = +165$. IR (neat) 2969, 1585, 1106, 1064, 1039, 956 cm$^{-1}$; 7.4 - 7.2 (m, 5 H), 6.8 (d, $J = 11.0$ Hz, 1 H), 6.5 (d, $J = 11.0$ Hz, 1 H), 6.4 (overlapping dd, $J = 11.0$ Hz, 2.0 Hz, 2 H), 6.4 (d, $J = 6.5$ Hz, 1 H), 3.4 (s, 3 H), 3.3 (3.3, 3 H), 1.5 (d, $J = 6.5$ Hz, 3 H); HRMS Calcd for C$^{16}$H$^{19}$O$^2$N: m/e 257.1415, obsd 257.1415.

Preparation of 62

$N$-Acetyl-$p$-benzoquinone imine dimethyl ketal (150 mg, 0.77 mmol) was dissolved in THF (10 mL), 1.0 N NaOH solution (5 mL) added, and the heterogeneous mixture stirred vigorously at room temperature for 1.0 h. R-$\alpha$-Methyl benzylamine (0.150 mL, 1.1 mmol) was added and the mixture stirred for an additional 6.0 h. The solution was transferred to a separatory funnel and extractive workup performed with Et$\_2$O (2 x 25 mL) giving the crude product as a yellow oil (300 mg). The crude oil was dissolved in dry THF (5 mL), LiAlH$\_4$ (150 mg) added, and the mixture stirred vigorously at room temperature under nitrogen. After 5.0 h, H$_2$O (20 mL) was added, and extractive workup with CH$_2$Cl$_2$ (2 x 35 mL) gave a light brown oil (88 mg). The oil was impregnated on Florisil (2.0 g) and column chromatography performed with a Florisil support (15 cm x 2 cm column) using 10% EtOAc/H as eluant.
The product 62 was isolated as a clear colorless oil (105 mg, 60\% yield). IR (neat) 3403 (w), 2927, 1512, 1450, 1235, 1038, 819 cm\(^{-1}\); \(^1\)H NMR \(\delta\) 7.5 - 7.2 (m, 5 H), 6.6 (AB q, \(\Delta v = 66.0\) Hz, J = 9.0 Hz, 4 H), 4.5 (q, J = 7.0 Hz, 1 H), 3.7 (s, 3 H), 1.5 (d, J = 7.0 Hz, 3 H); HRMS Calcd for C\(_{15}\)H\(_{17}\)O: m/e 227.1306, obsd 227.1301.

List of References


64. An authentic sample of 24 was obtained via courtesy of Professor Michael Novak. The formation of 24 (\(R_f = 0.50\), EtOAc) was not observed by TLC analysis of the hydrolysis reactions of 44a in alkaline, acidic and neutral media.


66. Calder reported the 1,2-addition of aniline to \(N\)-acetyl-2,6-dimethyl-p-benzoquinone imine, see ref. 1b.


68. Beilstein IV, 6, 4600.

69. Beilstein IV, 12, 3223.
Kinetic Data
<table>
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<tr>
<th>Min</th>
<th>Abs (316)</th>
<th>Abs (310)</th>
<th>Prod Conc</th>
<th>A-X/A</th>
<th>-Ln(A-X)/A</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.921</td>
<td>4.59E-05</td>
<td>0.8511</td>
<td>0.0088</td>
</tr>
<tr>
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<td>1.025</td>
<td>6.27E-05</td>
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<td>0.1612</td>
</tr>
<tr>
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<td>1.116</td>
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<td>0.2275</td>
</tr>
<tr>
<td>55</td>
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<td>0.2891</td>
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<tr>
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</tr>
<tr>
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<td>0.4641</td>
</tr>
<tr>
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<td>0.5232</td>
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<tr>
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<tr>
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<td>1.721</td>
<td>1.75E-04</td>
<td>0.4325</td>
<td>0.7819</td>
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<tr>
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<td>1.353</td>
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<td>1.85E-04</td>
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<td>0.8382</td>
</tr>
</tbody>
</table>

**Figure 46. Phenyl Migration of 41a with 1.4 x 10^-4 M CF3CO2H**

(a) Kinetic Run #1; (b) First-Order Plot of Run #1.
(a)

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Abs (316)</th>
<th>Abs (310)</th>
<th>Prod Conc</th>
<th>A-X/A</th>
<th>-Ln(A-X)/A</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2.01E-05</td>
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</tr>
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<td>0.855</td>
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</tr>
<tr>
<td>30</td>
<td>0.674</td>
<td>0.931</td>
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<td>0.1677</td>
</tr>
<tr>
<td>40</td>
<td>0.731</td>
<td>1.003</td>
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<td>0.8083</td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>0.3425</td>
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<td>1.15E-04</td>
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</tbody>
</table>

(b)

Figure 47. Phenyl Migration of 41a with 1.4 x 10^-4 M CF3CO2H
(a) Kinetic Run #2; (b) First-Order Plot of Run #2.
### Table 1

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Abs(316)</th>
<th>Abs(310)</th>
<th>Prod Conc</th>
<th>A-X/A</th>
<th>-Ln(A-X)/A</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>5</td>
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<tr>
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<tr>
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<td>0.4087</td>
<td>0.8947</td>
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<td>0.000141</td>
<td>0.3354</td>
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</tr>
</tbody>
</table>

### Figure 48

Phenyl Migration of 41b with $1.4 \times 10^{-4} \text{ M CF}_3\text{CO}_2\text{H}$

(a) Kinetic Run #1; (b) First-Order Plot of Run #1.
(a)

<table>
<thead>
<tr>
<th>Min</th>
<th>Abs (316)</th>
<th>Abs (310)</th>
<th>Prod Conc</th>
<th>A-X/A</th>
<th>-Ln(A-X)/A</th>
</tr>
</thead>
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<td>0.0977</td>
</tr>
<tr>
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<td>0.659</td>
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</tr>
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<td>0.754</td>
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<td>0.7493</td>
<td>0.2886</td>
</tr>
<tr>
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<td>0.3557</td>
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<tr>
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</table>

(b)

![First-Order Plot of Run #2](image)

**Figure 49. Phenyl Migration of 41b with 1.4 x 10^-4 M CF₃CO₂H**

(a) Kinetic Run #2; (b) First-Order Plot of Run #2.
Figure 50. 200 MHz $^1$H NMR Spectrum of 41f
Figure 51. 200 MHz $^1$H NMR Spectrum of 44c
Figure 52. 200 MHz $^1$H NMR Spectrum of 45c
PLEASE NOTE:

Page(s) not included with original material and unavailable from author or university. Filmed as received.
Figure 54. 200 MHz $^1$H NMR Spectrum of 45d
Figure 55. 200 MHz $^1$H NMR Spectrum of 52
Figure 56. 50 MHz $^{13}$C NMR Spectrum of 52
Figure 57. 200 MHz $^1$H NMR Spectrum of 54
Figure 58. 200 MHz $^1$H NMR Spectrum of 56
Figure 59. 50 MHz $^{13}$C NMR Spectrum of 56
Figure 60. 200 MHz $^1$H NMR Spectrum of 57
Figure 61. 50 MHz $^{13}$C NMR Spectrum of 57
Figure 62. 200 MHz $^1$H NMR Spectrum of 58
Figure 63. 50 MHz $^{13}$C NMR Spectrum of 58
Figure 64. 200 MHz $^1$H NMR Spectrum of 59
Figure 65. 50 MHz $^{13}$C NMR Spectrum of 59
Figure 66. 200 MHz $^1$H NMR Spectrum of 60
Figure 67. 50 MHz $^{13}$C NMR Spectrum of 60
Figure 68. 200 MHz $^1$H NMR Spectrum of 61
Figure 69. 200 MHz $^1$H NMR Spectrum of 62
Chapter IV

The Reactivity of N-Acylated p-Benzoquinol Ether Imines with Ethanethiol: A Model Study for the Incorporation of Glutathione during the Metabolism of Phenacetin and N-Acetyl-2-Aminofluorene.

Introduction

The metabolism of most drugs and xenobiotics generally begins with activation of the agent to a more polar species to facilitate excretion. However, this physiological process can generate highly reactive intermediates which if not deactivated can cause serious cellular damage. Fortunately, plants and animals possess relatively high concentrations of the biological thiol, glutathione, which acts to detoxify such intermediates. Due to the free sulfhydryl group present in the tripeptide backbone, glutathione can function in the dual role as a reducing agent for reactive oxidative species such as organic hydroperoxides or as a nucleophile which binds to reactive electrophilic intermediates.

Extensive investigations on the mechanistic aspects of acetaminophen metabolism have contributed greatly to the understanding of the pathological function of glutathione detoxification. Studies have shown acetaminophen is primarily metabolized to its glucuronide and sulfate conjugates and via cytosolic enzymatic processes which are then excreted in the urine along with the parent drug (Scheme XXXV). However, a fraction of the drug is oxidized by
cytochrome P-450 oxidase present in the endoplasmic reticulum which forms a chemically reactive intermediate that covalently binds glutathione preferentially. This adduct is further metabolized and excreted in the urine as the cysteiny1 conjugate 65 and the N-acetyl-cysteiny1 conjugate 66 (mercapturic acid adduct). Numerous investigations have linked the formation of these thiol-adducts with the reactive intermediate NAPQI 3, an extended π-bonded system that would be expected to react with soft, polarizable nucleophiles such as thiols.  

Interestingly several different thiol groups have been shown to inhibit protein alkylation during acetaminophen metabolism. As illustrated in Table 12, thiols added to microsomal incubations of
acetaminophen were shown to inhibit protein binding by forming covalently linked adducts and had varying reactivity toward the alkylating metabolite.\textsuperscript{73} Interestingly glutathione exhibits the greatest efficiency for adduct formation with almost an order of magnitude greater than \textit{N}-acetyl-cysteine which is the treatment given to patients of acetaminophen overdose.\textsuperscript{74}

In addition this study indicates the reactivity of protein sulfhydryl groups (estimated to have a concentration of 150 nm/mL) are comparable to the reactivity of the added glutathione (100 nm/mL) for adduct formation. However in vivo studies have shown significant alkylation of hepatic protein does not occur until about 75\% of the tissue glutathione has been depleted.\textsuperscript{75} Thus the incredible efficiency of which glutathione inhibits alkylation of protein sulfhydryl residues has been attributed to the action of glutathione-S-transferase. Interestingly addition of an active cytosolic transferase system to the microsomal incubation of acetaminophen almost completely inhibited covalent binding of acetaminophen metabolites to protein sulfhydryl groups with a glutathione concentration of 500 nmol/mL (Table 13).\textsuperscript{76} In addition prior denaturation of the cytosolic fraction did not effect the the adduct/protein bound ratios significantly for cysteine or \textit{N}-acetylcysteine indicating these thiols are not effective substrates for glutathione-S-transferase. Thus glutathione-S-transferase appears to play an integral part in the detoxification of metabolites during acetaminophen metabolism.
### Table 12. Effect of Thiols on Protein Alkylation during Acetaminophen Metabolism.

<table>
<thead>
<tr>
<th>Nucleophile (100 nmol)</th>
<th>Acetaminophen Metabolized (nmol 10 min⁻¹)</th>
<th>Metabolite Bound to Protein (nmol 10 min⁻¹)</th>
<th>Metabolite–Nucleophile Adduct (nmol 10 min⁻¹)</th>
<th>mmol Adduct Bound to Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>None added</td>
<td>9.6</td>
<td>7.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.8</td>
<td>7.6</td>
<td>0.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Cysteine</td>
<td>7.0</td>
<td>3.8</td>
<td>0.4</td>
<td>0.57</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>10.0</td>
<td>2.8</td>
<td>1.6</td>
<td>1.00</td>
</tr>
<tr>
<td>Glutathione</td>
<td>9.4</td>
<td>2.6</td>
<td>1.6</td>
<td>0.92</td>
</tr>
<tr>
<td>α-Mercaptopropionylglycine</td>
<td>8.6</td>
<td>2.4</td>
<td>0.5</td>
<td>0.35</td>
</tr>
<tr>
<td>Cysteamine</td>
<td>4.4</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 13. Effect of Glutathione-S-Transferase on Protein Alkylation during Acetaminophen Metabolism.

<table>
<thead>
<tr>
<th>Microsomal Protein mg Protein</th>
<th>Hepatic Cytosol mg Protein</th>
<th>Added Nucleophile (nmol)</th>
<th>RS Adduct (nmol)</th>
<th>Metabolite Bound Covalently (nmol)</th>
<th>mmol Adduct Bound to Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 150b</td>
<td>1.33</td>
<td>GSH (50)</td>
<td>3.90</td>
<td>0.95</td>
<td>4.11</td>
</tr>
<tr>
<td>2 150b</td>
<td>1.33</td>
<td>(100)b GSH (50)</td>
<td>0.80</td>
<td>1.21</td>
<td>0.66</td>
</tr>
<tr>
<td>2 150b</td>
<td>1.33</td>
<td>GSH (500)</td>
<td>5.00</td>
<td>0.01</td>
<td>5.00</td>
</tr>
<tr>
<td>2 150b</td>
<td>1.33</td>
<td>(100)b GSH (500)</td>
<td>3.00</td>
<td>0.35</td>
<td>8.57</td>
</tr>
<tr>
<td>2 150b</td>
<td>1.33</td>
<td>Cysteine (500)</td>
<td>1.40</td>
<td>0.24</td>
<td>5.83</td>
</tr>
<tr>
<td>2 150b</td>
<td>1.33</td>
<td>N-Acetylcysteine (500)</td>
<td>1.20</td>
<td>0.20</td>
<td>6.00</td>
</tr>
</tbody>
</table>

*Thiol content of protein fraction estimated from the milligram amounts of protein added and a value of 75 nmol of PSH per milligram of hepatic protein. 

† Cytosolic fraction heat denatured prior to use.
In contrast to acetaminophen, the pathological detoxification of the carcinogen N-acetyl-2-aminofluorene 8 is not as well understood. As described in Chapter 1, the current body of evidence supports the hypothesis that 2-AAF is initially N-hydroxylated, followed by a second activation step in the cytosol to generate the activated ester, AAF-N-sulfate 19. Novak has shown that AAF-N-sulfate 19 rapidly decomposes in water to form the p-benzoquinol imine 22 which kinetic data support as a nitrenium ion generated intermediate. Depending on the pH conditions, 22 may then react with water or Cl\(^-\) to form either the ortho chloro-amide 21 or the 4-hydroxy-amide 24. In addition, Meerman et al. investigated the decomposition of N-acetoxy-AAF 15 in a buffered phosphate solution at 37 °C in the presence of 10 mM concentration of glutathione and reported the formation of the two ortho-substituted glutathione conjugates 10 and 11 in 10% and 7% yields, respectively, as well as the isolation of the 4- and 7-substituted conjugates 16 and 17 (see Chapter 1). As mentioned previously, the 1- and 3-substituted glutathione-adducts 10 and 11 have been isolated from the urine of the rat whereas the formation of the adducts 16 and 17 have not been observed in test animals.

The authors proposed the formation of the conjugates 10, 11, 16, and 17 as occurring via glutathione capture of the nitrenium-ion generated intermediate 20 (Scheme XXXVI). For example, the formation of 10, 11, and 17 can be explained by glutathione capture of the charge-delocalized intermediates at the 1, 3, and 7-positions of the aromatic system followed by aromatization. Interestingly the authors
rationalized the formation of the 4-substituted glutathione conjugate 16 via initial para capture of the intermediate 20, followed by 1,2-migration of the glutathione moiety to form 16.

In support of Meerman's mechanistic hypothesis, investigations by McClelland have established that free nitrenium ions can be preferentially captured by nucleophiles in aqueous media. From these
studies, McClelland reported the free nitrenium-ion 68 of
N-(2,6-dimethylphenyl)hydroxylamine 67 in $3 \times 10^{-4}$ HClO$_4$ solution
reacted with water with a rate constant of $7 \times 10^8$ s$^{-1}$ forming the
Bamberger rearrangement product 69 in 96% yield (Scheme XXXVII). However a mixture of 69 and the azide 70 was obtained when the
decomposition of 67 was performed in azide buffer (pH 4.0), and
interestingly, the relative yield of 69 and 70 was reported to be a
function of azide concentration (Table 14). For example with a 0.05 M
centration of azide, 69 was formed in 71% yield relative to a 23% yield of 70 whereas a 0.4 M concentration of azide dramatically
increased the yield of 70 (73%) at the expense of 69 (19%). In
addition, McClelland calculated the rate of reaction of azide with the nitrenium ion 68 as diffusion-limited, e.g., $k_{az} = 5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$.

Table 14. Effect of Azide Concentration on Decomposition of 67.$^{80}$

<table>
<thead>
<tr>
<th>[N$_3^-$]</th>
<th>% Yield</th>
<th>69</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 M</td>
<td></td>
<td>75</td>
<td>23</td>
</tr>
<tr>
<td>0.10 M</td>
<td></td>
<td>55</td>
<td>42</td>
</tr>
<tr>
<td>0.20 M</td>
<td></td>
<td>28</td>
<td>59</td>
</tr>
<tr>
<td>0.40 M</td>
<td></td>
<td>19</td>
<td>73</td>
</tr>
</tbody>
</table>

Thus the investigations by McClelland have provided strong evidence that powerful nucleophiles can capture nitrenium ion intermediates with sufficient concentrations of the nucleophile in aqueous media. However the concentration of glutathione, which would have a similar nucleophilicity to azide according to Pearson's investigations,$^{81}$ has been estimated to be 6 - 8 mM in hepatic tissue.$^{70d}$ At these concentrations, the in vivo capture of the nitrenium ion 20 by glutathione becomes extremely unlikely and further emphasizes the importance of the metabolic intermediate 22. Therefore Chapter IV will describe the investigations on the reactivity of the $N$-acylated $p$-benzoquinol ether imine analogues of 22 with ethanethiol...
as a model study for the incorporation of glutathione during the metabolism of N-acetyl-2-aminofluorene 8.
Results

Initial studies on the incorporation of sulfur nucleophiles during the metabolism of N-acetyl-2-aminofluorene were directed at investigating the formation of the 4-substituted glutathione conjugate 16. Meerman et al. proposed the formation of 16 occurred via initial generation of the intermediate 20-V followed by a 1,2-shift and aromatization (Scheme XXXVI). Although they have not been reported for quinone systems, attempts were undertaken to synthesize an alkyl sulfur linkage in the 4-position of an N-acylated p-benzoquinone imine ketal derivative.

The most feasible approach appeared to be the synthesis of a mixed oxygen-sulfur ketal of an acylated p-benzoquinone imine derivative since the formation of the mixed ketal moiety from a dialkyl ketal was a well documented transformation. However attempts to prepare these compounds by an acid catalyzed exchange reaction with ethanethiol were unsuccessful. For example, addition of a catalytic amount of p-TsOH with EtSH (1.0 eq) to a solution of 32a in CH₂Cl₂ at 0 °C for 3 minutes gave the thiol-adducts 71 and 72 in 92% and 8% yields, respectively (Scheme XXXVIII). In addition, manipulation of reaction variables such as lower reaction temperatures, and addition of excess ethanethiol, gave similar results.

Therefore a different approach was examined, e.g., protection of the acylated quinol imine, ketal exchange, and then deprotection to regenerate the p-benzoquinone imine ketal. Initial efforts with this strategy were performed with the adduct 31a which was easily prepared
from the anodic oxidation of 30a (see Introduction of Chapter 2). However addition of a catalytic amount of pTsOH with EtSH (1.0 eq) to 31a in CH₂Cl₂ at 0 °C for 5 minutes gave a curious result, e.g., isolation of the p-benzoquinone imine ketal 32a (60%) in addition to the thiol-adducts 71 and 72 in 20% and 5% yield, respectively (Scheme XXXIX). Apparently with compounds such as 31a, the mixed methoxy-amide ketal ionizes more readily relative to the dimethyl ketal in mild acidic conditions, and prompted the investigation : a less basic protecting moiety for p-benzoquinone imine ketals.

According to the literature, the preparation of α-aminonitriles was a proven method for the protection of imines, and therefore was examined in the acylated quinone imine ketal system. Surprisingly the
Scheme XXXX.

reaction of 32a with potassium cyanide using 18-crown-6 as catalyst in THF at room temperature gave the 1,2-addition product 73 in 77% yield (Scheme XXXX). To demonstrate the feasibility of the cyano group as a method for protecting the imine linkage in p-benzoquinone imine ketals, the exchange of the dimethyl ketal of 73 was exchanged for the ethylene ketal by reaction with ethylene glycol and p-TsOH in THF. A simple deprotection of the product 74 with potassium t-butoxide at room temperature then gave the p-benzoquinone imine ethylene glycol ketal 75 in 65% overall yield. This reaction prepares for the first time the ethylene glycol ketal of an acylated p-benzoquinone imine, compounds not available via electrochemical oxidation methodology (see Chapter 2). To demonstrate the synthetic utility of this reaction, an additional example was performed with the acylated quinone imine 76
and gave the ethylene glycol ketal quinone imine 79 in 48% over 3 steps.

Having established conditions for the exchange reaction with oxygen nucleophiles, 73 was reacted with ethanethiol with acid-catalysis to form the mixed ketal 80 (Scheme XXXXI). However,

![Scheme XXXXI.](image)

deblocking of 80 with potassium t-butoxide under a variety of reaction conditions did not lead to the expected mixed ketal of the acylated quinone imine, 81. Instead the meta-substituted thiol-amide 72 was formed as the exclusive product in 66% yield. Since 75 and 79 were cleanly prepared from 73 and 76 under these reaction conditions, it appears that 72 results from an extremely rapid 1,2-migration of the thioethyl group in 81.

To demonstrate that the formation of 72 was in fact due to the rapid rearrangement of 81 and not due to adventitious acid in the work-up, the deblocking of 80 was followed by $^1$H NMR (Figure 70). Interestingly, upon addition of potassium t-butoxide to 80 in THF-d$_6$.
Figure 70. Deprotection of 80 followed by 250 MHz $^1$H NMR: (a) before addition of potassium t-butoxide; (b) after 2 min. at RT; (c) after 2 h at -78 °C; (d) 5 min after being warmed to RT.
the formation of the elusive intermediate 81 was observed by 250 MHz $^1$H NMR. For example after 2 h at -78 °C, complete consumption of the 79 was observed giving a mixture of 81 and and the thiol rearrangement product 72. However when the mixture was allowed to warm to room temperature, complete rearrangement of 81 was observed after 5 minutes.

The results of this experiment establishes the extremely facile 1,2-shift of an thioethyl group in the acylated quinone imine ketal 81. Thus, if products akin to 81 are formed in the metabolic processes of acylated aromatic amines such as phenacetin, a 1,2 shift of a sulfur group may be a viable process for the incorporation of sulfur based nucleophiles. In addition this investigation confirms in part the hypothesis by Meerman et al, that the formation of the glutathione-conjugate 16 may in fact follow initial capture of the nitrenium ion 20 to form the intermediate 20-V which subsequently rearranges to give 16.

In addition to the above investigations, a brief study was undertaken to determine the migratory aptitude of a 4-substituted thio group tethered to the p-benzoquinone ring. The synthetic strategy was very similar to the one used above; addition of 2-mercaptoethanol (10 eq) added to a solution of 73 with a catalytic amount of $p$-TsOH. After 15 minutes at 0 °C, the reaction was quenched with NaHCO$_3$ and upon isolation, gave a low yield of 82 (15%) and unfortunately, efforts to improve the yield of 82 were unsuccessful (Scheme XXXXII). The deprotection step was performed as described earlier with potassium...
c-butoxide at room temperature and gave 83 in 80% yield. Thus tethering the thio-group of an N-acylated p-benzoquinone imine ketal does not prevent the facile 1,2-shift of the sulfur moiety.

With the migratory aptitude of 4-substituted ethylthiol-p-benzoquinone imine ketals fully investigated, further effort was directed at investigating the reactivity of acylated quinol ether imines with alkylthiol groups. Initial model studies began with the addition of ethanethiol to a methanolic solution of 32a. Interestingly after 3.0 h at room temperature, 32a appeared to be completely inert. However when the solution was heated to 55 °C for 4.0 h, work-up and isolation gave an 89% yield of the ortho-substituted amide 71 and a 11% yield of the meta-substituted amide 72 (Scheme XXXXIII). Since the ortho-substitution product was analogous to that observed in the metabolism of N-acetyl-2-aminofluorene 8 this reaction was studied in more detail. As mentioned earlier, addition of ethanethiol to 32a with acid catalysis at 0 °C gave an almost identical product ratio of 71 and 72 and further investigations were pursued along this line. Surprisingly addition of 32a to a solution of ethanethiol containing a catalytic amount of p-TsOH at -78 °C, followed by quenching of the
reaction with sodium bicarbonate after two minutes led to a mixture of 32a and a new product identified as 84 (Scheme XXXXIII). Although 84 is labile, it was obtained in 51% yield (>95% pure by 1H NMR) from the reaction mixture by careful fractional recrystallization, mp 103.0 - 104.0 °C. The infrared spectrum of 84 indicated the presence of the secondary amide (1645, 1525, and 3295) and the 1H NMR (250 MHz) established the structure: δ 7.8 - 7.6 (m, 2 H), 7.5 - 7.3 (m, 3 H), 6.27 (br s, 1 H), 6.17 (AB q, J = 10 Hz, Δν = 27 Hz, 4 H), 3.35 (s, 3 H), 3.31 (s, 3 H), 2.49 (q, J = 7 Hz, 2 H), 1.21 (t, J = 7 Hz, 3 H).

Most importantly the ethanethiol adduct 84 rearranges slowly to 71 upon standing or rapidly gives 71 with acid catalysis. In addition, performing the acid-catalyzed conversion of 84 to 71 in the presence of a 10-fold molar excess of 1-propanethiol resulted in no incorporation
Figure 71. 200 MHz $^1$H NMR Spectrum of 84
of the propanethiol group supporting the intramolecularity of the 84 to 71 transformation. **This experiment demonstrates for the first time a 1,2-adduct of a thio group of an acylated quinone imine ketal and the facile 1,2-migration of the thio group with formation of an ortho-substituted amide.**

Since chemistry analogous to that described above would account for the formation of glutathione conjugates at the 1- and 3-positions of 8, further efforts were focused on the reactivity of the p-benzoquinol ether imine 44a with ethanethiol. Using the reaction conditions described above, ethanethiol was added to a methanolic solution of 44a and the solution heated at 55 °C for 3.0 h (Scheme XXXIV above). Interestingly, work-up and isolation of the reaction mixture gave two components which were easily separable by flash
column chromatography. The first component A, consisted of two thio-adducts which were separated by reverse-phase HPLC and characterized as 1-ethylthio-AAF 85 and 3-ethylthio-AAF 86. In addition, calculation of the isolated yields from the peak integrals of the 1H NMR spectra of component A, gave 85 and 86 in 38% and 23% yield, respectively. Analysis of the second component B consisted of one thio-adduct which was characterized as 4-ethylthio-AAF 87 and isolated in 13% yield.

The position of substitution on the fluorene nucleus was established from the 1H NMR spectra of each thio-adduct. In the NMR spectra of 85, an AB coupling pattern was observed which would only be consistent with substitution at the 1-position. In addition an 0.8 ppm downfield shift of H3 was observed for 85, a phenomenon observed in ortho-substituted amides.86 For 86, two meta-coupled doublets were observed with H1 deshielded 1.0 ppm and H4 shifted downfield by 0.3 ppm. The aromatic fingerprint of 87, initially, was not as intuitively easy to interpret. In the 1H NMR spectra of 87, a deshielded doublet was observed at 8.5 ppm along with a meta-coupled doublet at 7.6 ppm. According to the literature, the 1H NMR spectra of K-region substituted tert-butylthio-polyaromatics exhibit a large deshielded peri-proton effect.87 Thus the resonance at 8.5 ppm is consistent with the peri-proton H5 of the fluorene nucleus and the resonance at 7.6 ppm consistent with H1. In addition authentic sample
Figure 72. 200 MHz $^1$H NMR Spectrum of 85
Figure 73. 200 MHz $^1$H NMR Spectrum of 86
Figure 74. 200 MHz $^1$H NMR Spectrum of 87
of 87 was prepared by the addition of sodium ethylthiol to 44a at room
temperature in THF. The 1,4-addition product 87 was formed in 75%
yield and all spectra consistent with the designated structure.

In summary, this investigation has shown that the production of
the 1- and 3-substituted glutathione-conjugates during the metabolism
of N-acetyl-2-aminofluorene 8 in the rat may be generated by
glutathione capture of the N-acetyl-p-benzoquinol imine 22, and in
view of the relatively low concentrations of glutathione in hepatic
tissue, casts strong doubt on the alternative hypothesis of
nitrenium-ion capture to generate such adducts (see Scheme XIII, p
25). In addition, the results presented herein provide strong
evidence that the nitrenium-ion generated p-benzoquinol imine 22 may
in fact be the "ultimate" carcinogenic metabolite of
N-acetyl-2-aminofluorene 8 metabolism. With these results in mind,
further investigative efforts on the reactivity of N-acylated
p-benzoquinol imines with glutathione and nucleic acids under
physiological conditions must be undertaken to fully understand the
metabolic role of 22.
Experimental

General Procedure

Melting points were determined in capillaries in a Thomas-Hoover "Unimelt" apparatus and are uncorrected. IR spectra were determined on a Perkin-Elmer Model 283B spectrometer. Unless noted otherwise, the $^1$H NMR spectra were measured on a Bruker 200 MHz $^1$H NMR using deutereochloroform as solvent and residual chloroform as standard. Mass spectral and exact mass measurements were obtained by Mr. Richard Weisenberger on a Kratos MS-30 spectrometer connected to a DS-55 data system. Combustion analyses were performed by Scandinavian Microanalytical Laboratory, Herlev, Denmark, and M-H-W Laboratories, Phoenix, Arizona. All reagents or compounds not explicitly referenced were obtained from the Aldrich Chemical Co. Alumina and silica gel (Kieselgel 60 230-400 mesh) were obtained from E. Merck Co and Florisil (100 - 200 mesh) purchased from Fischer Scientific Co. Thin-layer chromatography (TLC) was done using Merck silica gel 60 F$_{254}$ pre-coated aluminum backed plates, 0.2-mm thickness. Visualization was by UV or by spraying with 5% ethanolic phosphomolybdic acid and then heating. Tetrahydrofuran was purified by distillation from benzophenone ketyl and methanol purified by distillation from Mg/I$_2$ and the middle fraction collected. Throughout the Experimental Section the following abbreviations are used: petroleum ether, bp 35-60 °C (PE), hexanes, bp 68-69 °C (H), ethyl acetate (EtOAc), tetrahydrofuran
(THF), p-toluene sulfonic acid (p-TsOH), ethanethiol (EtSH) and thin layer chromatography (TLC). Extractive workup refers to extraction of the material into the indicated solvent, washing the organic layer with brine solution, drying over Drierite (CaSO₄), concentration in vacuo, and drying to constant weight under vacuum (1-2 Torr).

**1H NMR Studies.** The starting amide 73 (25 mg, 0.08 mmol) and potassium t-butoxide (10 mg, 0.09 mmol) were placed in a dry NMR tube and the contents placed under vacuum for 5 minutes. Dry THF-d₆ (1 mL) was then added and the mixture shaken vigorously until a dark yellow color prevailed and a 1H NMR (250 MHz) taken of the reaction mixture over a two minute period at room temperature (Figure 70b). The NMR tube was then placed in a dry ice/acetone bath for 2.0 h., followed by additional scanning of the cooled reaction mixture (Figure 70c). After 3 minutes the reaction mixture was warmed to room temperature and scanning continued which showed complete consumption of the p-benzoquinone imine 74 (Figure 70d).

**Preparation of 73**

*73* N-Benzoyl-p-benzoquinone imine

dimethyl ketal (500 mg, 1.94 mmol) and potassium tert-butoxide (1.2 g, excess) and 18-Crown-6 (710 mg, 1.4 eq) were
dissolved in dry THF (15 mL) under nitrogen. The reaction mixture was stirred at RT for 0.5 h. Wet THF (5 mL) was then added and the solvent removed in vacuo to yield a dark green residue. Water (20 mL) was added and after 20 min a white precipitate formed. Filtration of the precipitate yielded a greenish white crystalline solid (482 mg); mp 138-142 °C. A recrystallization from CH₂Cl₂/H₂O gave a white solid (429 mg, 78% yield), mp 146-147 °C. After two recrystallizations analytically pure material was obtained, mp 148.0 - 148.5 °C (lit. mp 145.0 - 147.0 °C) and spectral properties corresponded to authentic sample. IR (KBr) 1655, 1510, 1480, 1110, 1070, 1040, 960, 710 cm⁻¹; ¹H NMR δ 7.8-7.5 (m, 2 H), 7.5-7.2 (m, 3 H), 6.9 (br s, 1 H), 6.5 (AB q, J_AB = 10 Hz, Δv = 24 Hz, 4H), 3.3 (s, 3H), 3.2 (s, 3 H); ¹³C NMR δ 167, 132 (2C), 130 (2C), 128 (2C), 126 (2C), 116, 92, 50, 49, 46.

Preparation of 74

4-(N-Benzyloxy)-4-cyano-2,5-cyclohexadienone dimethyl ketal (50 mg, 0.83 mmol) and a catalytic amount of p-TsOH (15 mg) were dissolved in dry ethylene glycol (7 mL, excess) under nitrogen. Dry THF (5 mL) was then added to afford a homogeneous solution and stirred for 2.5 h at RT. NaHCO₃ (1 mL) was added and the solvent removed in vacuo. H₂O was then added to the residue
which formed a white precipitate. After 45 min the precipitate was filtered and dried yielding a white solid (180 mg, 77%), mp 195-197 °C. A recrystallization from Et₂O/H yielded white crystals, mp 201-202 °C. IR (KBr) 3395, 2230 (w), 1660, 1510, 1480, 1140, 1120, 975, 710 cm⁻¹; ¹H NMR δ 7.8-7.7 (m, 2 H), 7.5-7.4 (m, 3 H), 6.3 (AB q, δv = 32 Hz, J = 10 Hz, 4 H), 6.25 (br s, 1 H), 4.0 (s, 4 H); exact mass calc for C₁₆H₁₄N₂O₃ calc 282.1001 expt 282.1038

**Preparation of 75**

4-(N-Benzoyl)-4-cyano-2,5-cyclohexadiene ethylene glycol ketal (171 mg, 0.61 mmol) and potassium t-butyloxide (69 mg, 1.0 eq) were dissolved in dry THF (10 mL). The reaction mix was stirred for 7 min at RT. H₂O (2 mL) was then added and extractive work-up (EtOAc, 80 mL) yielded a yellow oil (150 mg). A ¹H NMR of the crude product mixture showed product to be greater than 90% pure. Column chromatography was performed using silica gel (230 - 400 mesh, 2 g, 1 cm x 20 cm) and 2% EtOAc/CH₂Cl₂ as eluant. The first compound off the column was isolated yielding a yellow oil. Addition of cold ether and scratching with a spatula yielded a yellow solid (130 mg, 84%), mp 65.0 - 66.0 °C. A recrystallization from Et₂O/H at 0 °C yielded white crystals, mp 69.0 - 69.5 °C. IR (KBr) 1670, 1650, 1605, 1260, 1245, 1195, 1115, 1060, 970 cm⁻¹; ¹H
Preparation of 77

N-Benzoyl-3-methoxy-p-benzoquinone

imine dimethyl ketal (500 mg, 1.7 mmol), potassium cyanide (1.2 g, excess) and 18-Crown-6 (710 mg, 1.5 eq) were dissolved in dry THF (15 mL) under nitrogen at RT. The mixture was stirred for 0.5 h and quenched with wet THF (2 mL). The solvent was removed in vacuo and H₂O (10 mL) added to the residue. The mixture was swirled gently for a few min and allowed to sit for approximately 10 min. A yellow orange precipitate formed which was filtered and dried yielding a light orange solid (410 mg), mp 160-165 °C. This solid was then recrystallized for CH₂Cl₂/H yielding a white solid (250 mg), mp 170-171 °C. A recrystallization of the mother liquors yielded additional compound (white solid, 65 mg, 315 mg total, 60% yield), mp 168-170 °C. A recrystallization from CHCl₃/H yielded analitically pure material; mp 179-180 °C. IR (KBr) 3345, 2230 (w), 1655(sh), 1515, 1485, 1390, 1295, 1215, 1100, 960, 710 cm⁻¹; ¹H NMR δ 7.8-7.7 (m, 2 H), 7.6-7.4 (m, 3 H), 6.6 (dd, Jₐ₋ₜ = 10 Hz, 2 Hz, 1 H), 6.4 (bs, 1 H), 6.1 (d, Jₐ₋ₜ = 10 Hz, 1 H), 5.5 (d, J = 2 Hz, 1 H); HRMS Calcd for C₁₅H₁₃O₃N: m/e 255.0892, obsd 255.0904.
H), 3.7 (s, 3 H), 3.3 (s, 3 H), 3.3 (s, 3 H); HRMS Calcd for C$_{17}$H$_{18}$O$_4$N$_2$: m/e 314.1262, obsd 314.1278.

Preparation of 78

Ethylene glycol (10 mL) was added to 3-methoxy-4-(N-benzoylamino)-4-cyano-2,5-cyclohexadienone dimethyl ketal (300 mg, 0.96 mmol) and a catalytic amount of p-TsOH (10 mg) under nitrogen. Dry THF (12 mL) was then added to yield a homogeneous mixture and the solution was stirred for 1 h at RT. NaHCO$_3$ (2 mL) was then added with subsequent addition of sat. brine solution (25 mL). Extractive work-up (CH$_2$Cl$_2$, 100 mL) yielded a white foam (300 mg). Trituration with cold ether yielded a white solid (280 mg, 94%), mp 172-173 °C. A recrystallization from CH$_2$Cl$_2$/H yielded white crystals, mp 174-175 °C. IR (KBr) 3295, 2225(w), 1655, 1520, 1490, 1395, 1295, 1220, 1190, 1160, 1090, 980 cm$^{-1}$; $^1$H NMR $\delta$ 7.8-7.7 (m, 2 H), 7.6-7.3 (m, 3 H), 6.5 (dd, $J_{AB} = 10$ Hz, 2 Hz, 1 H), 6.3 (bs, 1 H), 5.9 (d, $J_{AB} = 10$ Hz, 1 H), 5.4 (d, J = 2 Hz, 1 H), 4.2-4.0 (m, 4 H), 3.7 (s, 3 H); HRMS Calcd for C$_{17}$H$_{18}$O$_4$N$_2$: m/e 312.1106, obsd 312.1126.
Preparation of 79

3-Methoxy-4-(N-benzoylamino)-4-cyano-2,5-cyclohexadienone ethylene glycol ketal (135 mg, 0.43 mmol) and potassium t-butyloxide (48 mg, 1.0 eq) were dissolved in dry THF (8 mL) under nitrogen. The mixture was stirred for 5 min at RT. H$_2$O (1 mL) was then added and the solvent removed in vacuo. The residue was extracted with CH$_2$Cl$_2$ (40 mL) which yielded a yellow oil (115 mg). A $^1$H NMR of the crude product mixture showed product to be greater than 90% pure. Column chromatography was performed using silica gel (2 g, 1 cm x 25 cm) and 2% EtOAc/H as eluant. The first compound off the column was isolated yielding a light yellow oil. Addition of cold ether and scratching with a spatula yielded a light yellow solid (100 mg, 85%), mp 98.0 - 99.5 °C. A recrystallization from Et$_2$O/H at 0 °C yielded white crystals, mp 103-104 °C. IR (KBr) 1680, 1655, 1600, 1445, 1280, 1260, 1205, 1160, 1075 cm$^{-1}$; $^1$H NMR δ 8.0-7.9 (m, 2 H), 7.6-7.3 (m, 3 H), 6.3 (AB q, Δv = 14 Hz, $J_{AB}$ = 10 Hz, 2 Hz, 2 H), 5.5 (d, 2 Hz, 1 H), 4.3-4.1 (m, 4 H), 3.7 (s, 3 H); HRMS Calcd for C$_{15}$H$_{15}$O$_4$N: m/e 285.0997, obsd 285.0995.
Preparation of 80

4-((N-Benzoylamino)-4-cyano-2,5-cyclohexadienone dimethyl ketal (200 mg, 0.7 mmol) was dissolved in dry THF (10 mL) and cooled to 0 °C under nitrogen for 20 min. A solution of p-TsOH (4.0 mg, 0.03 eq), ethanethiol (3.1 mL, excess), and dry THF (2 mL) was then added via syringe and the mixture stirred for 35 min. Addition of H₂O (30 mL) and extractive work-up (CH₂Cl₂, 2x30 mL) yielded a yellow oil (210 mg). Column chromatography was performed (12 g, 2 cm x 9 cm) using base-washed silica gel (5% NH₄OH/MeOH and dried under vacuum) and 2% EtOAc/CHCl₃ as eluant. The most polar spot by TLC was isolated yielding a light yellow solid (125 mg, 57%), mp 116-118 °C. A recrystallization from Et₂O/H₂O yielded clear colorless crystals, mp 110.5-111.0 °C. IR (KBr) 3280, 2230 (w), 1655, 1645, 1585, 1525 (sh), 1480, 1410, 1310, 1290, 1280, 1100, 1080, 890, 875 cm⁻¹; ¹H NMR (CDCl₃ (D₂O), 80 MHz) δ 7.8-7.7 (m, 2 H), 7.5-7.4 (m, 3 H), 6.34 (AB q, Δν = 16 Hz, J = 10 Hz, 4 H), 3.3 (s, 3 H), 2.6 (q, J = 7 Hz, 2 H), 1.2 (t, J = 7 Hz, 3 H); HRMS Calcd for C₁₇H₁₈O₂N₂S: m/e 314.1085, obsd 314.1117.

Anal. calcd for C₁₇H₁₈O₂: C, 64.94; H, 5.77. Found: C, 64.97; H, 5.80.
Deblocking of 80 with Potassium tert-Butoxide

4-(N-Benzyolamino)-4-cyano-2,5-cyclohexadienone ethylthiomethoxy ketal (100 mg, 0.318 mmol) and potassium tert-butoxide (36 mg, 1.0 eq) were dissolved in dry THF (7 mL) under nitrogen. The reaction mixture was stirred for 10 min at RT turning the clear colorless solution to a bright yellow. NaHCO₃ (1 mL) was then added and the solvent removed in vacuo. The residue was extracted with CHCl₃ (2x10 mL), the solvent dried over CaSO₄, and removed in vacuo yielding a pale orange solid (80 mg), mp 163-166 °C. A recrystallization from CH₂Cl₂/H yielded a white solid (60 mg, 66%), mp 181.0 - 182.0 °C (lit 80 mp, 185-185.5 °C). IR (KBr) 3290, 1640, 1590, 1575, 1515, 1485, 1460, 1450, 1400, 1310, 1255, 1240, 1225, 1070, 1020, 800 cm⁻¹; ¹H NMR (250 MHz) δ 7.8 (d, J = 8 Hz, 1H), 7.8 (s, 1H), 7.7 (bs, 1 H), 7.6-7.4 (m, 4 H), 7.4 (dd, J = 8 Hz, 2 Hz, 1 H), 6.8 (d, J = 8 Hz, 1 H), 3.9 (s, 3 H), 2.9 (q, J = 7 Hz, 2H), 1.3 (t, J = 7 Hz, 3 H).
Preparation of 82

4-(N-Benzoylamino)-4-cyano-2,5-cyclohexadienone dimethyl ketal (200 mg, 0.7 mmol) was placed in a dry 50 mL round-bottom flask, charged with stir bar and evacuated with nitrogen. Dry THF (6 mL) was then added and the solution placed in an ice-bath and stirred vigorously for 20 minutes. A solution of p-TsOH (30 mg, 0.16 mmol), 2-mercaptoethanol (0.4 mL, 6.0 mmol) and dry THF (2 mL) was then added with syringe and the reaction run at 0 °C. After 15 minutes NaHCO₃ (1 mL) was added and extractive work-up performed with CH₂Cl₂ (3 x 20 mL). Upon removal of solvent in vacuo, the residue was placed under vacuum (0.2 torr) for 3.0 h to remove excess 2-mercaptoethanol and give a clear colorless oil (215 mg). The oil was then dissolved in CH₂Cl₂ and impregnated on Florisil (1.0 g). Column chromatography was performed with a Florisil support (8 cm x 1 cm column) and 2% EtOAc/CHCl₃ (250 mL) used as eluant. The first compound off the column was isolated as a pale white solid (30 mg, 15% yield), mp 195.0 - 197.0 °C. A recrystallization from CH₂Cl₂/hexane gave a white crystalline solid, mp 203.0 - 205.0 °C. IR (KBr) 3307, 2230 (w), 1639, 1521, 1485, 1312, 1087, 900, 720 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) 7.8 (d, J = 7 Hz, 2 H), 7.5 - 7.2 (m, 3 H), 6.3 (s, 4 H), 4.3 (t, J = 6 Hz, 2 H), 3.2 (t, J = 6 Hz, 2 H); HRMS Calcd for C₁₆H₁₄O₂N₂S: m/e 298.0773, obsd 298.0765.
Preparation of 83

4-(N-Benzoylamino)-4-cyano-2,5-cyclohexadienone mercaptoethylene ketal (40 mg, 0.13 mmol) and potassium t-butoxide (15 mg, 0.13 mmol) were placed in a dry 25 mL round-bottom flask, charged with stir bar, and evacuated with nitrogen. Dry THF (4 mL) was added and the reaction mixture stirred vigorously for 9 min at room temperature. Water (2 mL) was then added, and extractive work-up (CH₂Cl₂, 2 x 15 mL) gave a cloudy white oil (40 mg). Column chromatography was performed with a Florisil support (10 cm x 1 cm column) and 2% EtOAc/CHCl₃ used as eluant. The first compound eluted from the column was a white crystalline solid (28 mg, 80% yield), mp 144.0 - 145.0 °C. IR (KBr) 3314, 1649, 1529, 1492, 1307, 1210 cm⁻¹; ¹H NMR δ 7.9 - 7.8 (m, 3 H), 7.6 - 7.4 (m, 4 H), 7.1 (dd, J = 10 Hz, 1.5 Hz, 1 H), 6.8 (d, J = 10 Hz, 1 H), 4.4 (t, J = 7 Hz, 2 H), 3.1 (t, J = 7 Hz, 2 H); HRMS Calcd for C₁₅H₁₃O₂NS: m/e 271.0664, 271.0665.
Preparation of 84

*N*-Benzoyl-\(p\)-benzoquinone imine dimethyl ketal (30 mg, 0.116 mmol) was dissolved in dry THF (0.6 mL) and cooled to -78 °C under nitrogen for 15.0 min. Ethanethiol (9 µL, 1.0 eq) was then added via syringe followed by the addition of a solution of \(p\)-TsOH (2 mg) and dry THF (0.3 mL). The reaction mixture was stirred for 2.0 min at -78 °C. \(\text{NaHCO}_3\) (1.0 mL) quenched the reaction and extractive work-up (\(\text{CH}_2\text{Cl}_2\), 2 x 10 mL) yielded a yellow oil. The oil was then pumped on under vacuum (0.2 torr) for 0.5 h yielding a yellow oily solid (30 mg). Trituration with hexane followed by a recrystallization with \(\text{Et}_2\text{O/H} (1.0/3.5 \text{ mL})\) on dry-ice gave a white solid (19 mg, 51%), mp 103-104 °C: IR (KBr) 3295, 1645, 1575, 1525, 1400, 1320, 1150, 1100, 1070, 970 cm\(^{-1}\); \(^1\)H NMR (250 MHz) \(\delta\) 7.73-7.69 (m, 2 H), 7.49-7.36 (m, 3 H), 6.27 (bs, 1 H), 6.18 (AB q, \(\Delta v = 27 \text{ Hz}, J_{AB} = 10 \text{ Hz}, 4 \text{ H})\), 3.35 (s, 3 H), 3.31 (s, 3 H), 2.49 (q, \(J = 7 \text{ Hz}, 2 \text{ H})\), 1.22 (t, \(J = 7 \text{ Hz}, 3 \text{ H})\);

HRMS Calcd for \(\text{C}_{17}\text{H}_{21}\text{O}_{3}\text{NS}\): \(m/e\) 319.1237, obsd 287.1010 (corresponds to parent ion peak - \(\text{CH}_3\text{OH}\)).
Acid-Catalyzed Rearrangement of 84

The ketal 84 (30 mg, 0.090 mmol) and p-TsOH (5 mg) were dissolved in dry THF (1 mL) under nitrogen. The reaction mixture was stirred for 15 min at room temperature. NaHCO₃ (0.5 mL) was then added and the solvent removed in vacuo. Extractive work-up gave an off-white solid (24 mg) which was predominantly 71 by TLC. Column chromatography using flash silica gel (200 mg, 5 cm x 0.5 cm) and CHCl₃ as eluant was performed. The first compound isolated off the column was a white solid (18 mg, 90%): mp 147-148.5 °C (lit. mp 147-147.5 °C). IR (KBr) 3260, 1645, 1515, 1485, 1300, 1215, 1060, 710 cm⁻¹; ¹H NMR (250 MHz) 8 9.1 (br s, 1 H), 8.5 (d, J = 9 Hz, 1 H), 7.9 - 7.8 (m, 2 H), 7.6 - 7.5 (m, 3 H), 7.1 (d, J = 3.0 Hz, 1 H), 6.9 (dd, J = 9.0, 3.0 Hz, 1 H), 3.8 (s, 3 H), 2.8 (q, J = 7.5 Hz, 2 H), 1.2 (t, J = 7.5 Hz, 3 H).

Addition of Ethanethiol to 32a at 55 °C

N-Benzoyl-p-benzoquinone imine dimethyl ketal (100 mg, 0.39 mmol) was dissolved in distilled CH₃OH (3 mL) in a 25 mL round-bottom flask, equipped with a condenser and heated to 55 °C. The reaction was monitored by TLC (40% EtOAc/H) and complete after 3.0 h. The reaction solvent was then removed in vacuo giving a yellow-white solid (120 mg) as crude product. Purification was performed via flash column chromatography (15 cm x 2 cm column) and
20% EtOAc/H used as eluant. The product 71 (R\textsubscript{f} = 0.71, 40% EtOAc/H) was the first component isolated as a white solid (100 mg, 89% yield), mp 144.0 - 146.0 °C (lit.\textsuperscript{80} mp 147.0 - 147.5 °C). The second component was characterized as 72 (R\textsubscript{f} = 0.57, 40% EtOAc/H) and isolated as a white solid (12 mg, 11% yield), mp 183.0 - 185.0 (lit.\textsuperscript{80} mp 185.0 - 185.5 °C).

**Addition of Ethanethiol to 44a at 55 °C**

The p-benzoquinol imine 44a (100 mg, 0.4 mmol) was dissolved in distilled CH\textsubscript{3}OH in a 25 mL round-bottom flask and equipped with condenser. Ethanethiol (1 mL, 14 mmol) was added and the solution heated to 55 °C. The reaction was monitored by TLC (40% EtOAc/H) and complete after 3.0 h. Extractive work-up was performed with CH\textsubscript{2}Cl\textsubscript{2} (3 x 20 mL), the organic layer washed with brine, and the solvent removed in vacuo giving a yellow solid (100 mg) as crude product. Purification was performed via flash column chromatography (15 cm x 2 cm column) and 25% EtOAc/H used as eluant. The first component (R\textsubscript{f} = 0.52, 40% EtOAc/H) eluted off the column was obtained as a white solid (70 mg). A \textsuperscript{1}H NMR spectra of component A determined it was a mixture of 1-ethylthio-AAF \textsuperscript{85} and 3-ethylthio-AAF \textsuperscript{86}. From the peak integrals, the isolated yields of \textsuperscript{85} and \textsuperscript{86} were calculated to be 23% and 39% yields, respectively. The second component eluted off the column was isolated as a light yellow solid (13 mg, 12% yield) and characterized as 87 (R\textsubscript{f} = 0.22,
40% EtOAc/H), mp 147.0 - 150.0 °C (see Preparation of 87 below for spectral properties).

An analytical sample of 85 was obtained via semi-preparative reverse-phase HPLC using a Rainin C_{18} 80-225-C5 semi-preparative column and 25% H_{2}O/CH_{3}OH solution as eluant and the least polar component of the HPLC trace collected. However due to the small quantities obtained by this method, spectral data of 85 was limited to a $^1$H NMR spectra: $\delta$ 8.8 (bs, 1 H), 8.5 (d, $J = 8.0$ Hz, 1 H), (overlapping doublets, 2 H), 7.5 (d, 7.0 Hz, 1 H), 7.4 - 7.2 (overlapping triplets, 2 H).

An analytical sample of 86 was obtained by three successive fractional recrystallizations of component A with CH_{3}OH/H_{2}O giving 86 as a white crystalline solid, mp 150.0 - 152.0 °C. IR (KBr) 1660, 1505, 1395 cm$^{-1}$; $^1$H NMR 8.7 (bs, 1 H), 8.6 (s, 1 H), 7.9 (s, 1 H), 7.7 (dd, $J = 7.0$ Hz, 1.5 Hz, 1 H), 7.5 (dd, $J = 7.0$ Hz, 1.5 Hz, 1 H), 7.3 - 7.2 (two overlapping triplets, 2 H); 3.9 (s, 2 H), 2.8 (q, $J = 7.0$ Hz, 2 H), 2.2 (s, 3 H), 1.2 (t, $J = 7.0$ Hz, 3 H); HRMS Calcd for C_{17}H_{17}ONS: m/e 283.1027, obsd 283.1029.
Preparation of 87 via Addition of Sodium Ethylthiol to 44a

Sodium hydride (60 mg, 60% dispersion) was placed in a 50 mL round-bottom flask, washed with hexane (2 x 4 mL), dry THF (3 mL) added, the suspension placed under nitrogen and ethanethiol (0.11 mL, 1.5 mmol) added via syringe causing immediate gas evolution. After bubbling ceased, a solution of the p-benzoquinone imine 44a (250 mg, 0.99 mmol) in dry THF (2 mL) was added via syringe and the mixture stirred vigorously at room temperature. The reaction was monitored by TLC (40% EtOAc/H) and appeared complete after 2.0 h. H₂O (10 mL) was added and extractive workup performed with CH₂Cl₂ (2 x 20 mL) giving a yellow oil (250 mg) as crude product. Purification was performed via flash column chromatography (15 cm x 2 cm column) and 20% EtOAc/H used as eluant. The product 87 (R_f = 0.22, 40% EtOAc/H) was isolated as a yellow solid (210 mg, 75% yield), mp 144.0 - 146.0 °C. A recrystallization from CH₃OH/H₂O gave analytically pure material as a light yellow solid, mp 147.0 - 148.5 °C. IR (KBr) 3320, 1665, 1580, 1540, 1460, 1400, 710 cm⁻¹;¹H NMR δ 8.4 (d, J = 7.0 Hz, 1 H), 7.6 (s, 1 H), 7.5 - 7.3 (m, 4 H), 3.8 (s, 2 H), 3.1 (q, J = 7.0 Hz, 2 H), 2.2 (s, 3 H), 1.4 (t, J = 7.0 Hz, 3 H); HRMS Calcd for C₁₇H₁₇O: m/e 283.1027, obsd 283.1031.
List of References


77. Taken in part from ref. 70c, p .

78. Taken in part from ref. 70c, p .


80. Taken in part from ref. 79.


83. The room temperature variant of this reaction was reported previously from these laboratories, see Swenton, J.S.; Bonke, B.; Clark, W.M.; Chen, C.P.; Martin, K.V. J. Org. Chem. 1990, 55, 2027.

85. Calder observed the addition of ethanethiol across the imine linkage of *N*-acetyl-2,6-dimethyl-p-benzoquinone imine whereas the 3,5-dimethyl derivative yielded 3′,5′-dimethyl-2′-(ethythio)-4′-hydroxyacetamide. He also proposed initial imine attack and migration to form the 2′-substituted adduct, however, this was not demonstrated experimentally, see ref. 1b.


Figure 75. 200 MHz $^1$H NMR Spectrum of 71
Figure 76. 200 MHz $^1$H NMR Spectrum of 72
Figure 77. 200 MHz $^1$H NMR Spectrum of 73
Figure 78. 50 MHz $^{13}$C NMR Spectrum of 73
Figure 79. 200 MHz $^1$H NMR Spectrum of 74
Figure 80. 200 MHz $^1$H NMR Spectrum of 75
Figure 81. 200 MHz $^1$H NMR Spectrum of 77
Figure 82. 200 MHz $^1$H NMR Spectrum of 78
Figure 83. 200 MHz $^1$H NMR Spectrum of 79
Figure 84. 200 MHz $^1$H NMR Spectrum of 80
Figure 85. 200 MHz $^1$H NMR Spectrum of 82
Figure 86. 200 MHz $^1$H NMR Spectrum of 83


19. See references 12 - 17 in 4c.


21. Extrahepatic toxicities associated with the overdose of phenacetin have not been reported for acetaminophen.


24. For an excellent review on the toxicity of N-acetyl-2-aminofluorene and other aromatic amines, see: Kriek, E. Biochimica et Biophysica Acta 1974, 355, 177.


35. Lotlikar, P.D. Xenobiotica 1971, 1, 543.


44. (a) Shono, T. Electroorganic Synthesis; Academic: San Diego, 1991. (b) Fry, A.J. Synthetic Organic Electrochemistry; Wiley-Interscience, New


49. Taken from ref. 48.


51. 2-Chloronaphthalene was not electrochemically stable under the reaction conditions. Therefore an aliquot (1 mL) of a 20 mM solution of 2-chloronaphthalene was added to an aliquot (1 mL) of reaction mixture prior to HPLC analysis.


53. Ibid, p. 182.


64. An authentic sample of 24 was obtained via courtesy of Professor Michael Novak. The formation of 24 (Rf = 0.50, EtOAc) was not observed by TLC analysis of the hydrolysis reactions of 44a in alkaline, acidic and neutral media.


66. Calder reported the 1,2-addition of aniline to N-acetyl-2,6-dimethyl-p-benzoquinone imine, see ref. 1b.


77. Taken in part from ref. 70c, p 7.

78. Taken in part from ref. 70c, p 9.


80. Taken in part from ref. 79.


83. The room temperature variant of this reaction was reported previously from these laboratories, see Swenton, J.S.; Bonke, B.; Clark, W.M.; Chen, C.P.; Martin, K.V. *J. Org. Chem.* 1990, 55, 2027.


85. Calder observed the addition of ethanethiol across the imine linkage of *N*-acetyl-2,6-dimethyl-p-benzoquinone imine whereas the 3,5-dimethyl derivative yielded 3',5'-dimethyl-2'-(ethylthio)-4'-hydroxyacetamide. He also proposed initial imine attack and migration to form the 2'-substituted adduct, however, this was not demonstrated experimentally, see ref. 1b.
