

**SYNTHESIS AND REACTIONS OF QUINONES**  
**WITH SOME POSSIBLE**  
**BIOLOGICAL APPLICATIONS**

by

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in

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## Synthesis and Reactions of Quinones with Some Possible Biological Applications

### ABSTRACT

The regioselective syntheses of various 5-acetoxy-2,3-disubstituted-1,4-naphthoquinone derivatives from 5-acetoxy-2-bromo-1,4-naphthoquinone with yields ranging from 80- 90% have been performed by modifying the Jacobsen-Thorsell oxidative substitution.

Interest in these types of quinones is emerging in several different fields. Since naphthoquinone derivatives have been shown to inhibit the HIV-1 reverse transcriptase (RT), some of the disubstituted naphthoquinones prepared in this work were synthesized to explore the nature of the quinone binding site on RT. Some of the compounds show interesting levels of anti-RT activity with 5-hydroxy-3-methyl-1,4-naphthoquinone being the most potent of the compounds studied.

A synthetic approach to the benzo[b]fluorene ring system of the kinamycins and related natural products, based on such a dialkylation sequence is proposed. Model studies concerning the stereoselective oxidative elaboration of the D-ring of the kinamycins and the regioselective introduction of the diazo group are described. Preliminary model studies concerning the construction of the A ring of the benzo[b]fluorene natural product cysfluoretin are also reported.

## **ACKNOWLEDGEMENTS**

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My thanks are also extended to my labmates and fellow graduate students who have made my stay in Waterloo a valuable experience on several levels.

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**To my family,**

***Lisette, Normand, Lisa-Renée, et Christian***

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## List of Abbreviations

3TC = 2',3'-dideoxy-3'-thiacytidine

a.a. = Amino acid

AIBN = 2,2'-azobisisobutyronitrile [ $\text{Me}_2\text{C}(\text{CN})\text{N}=\text{NC}(\text{CN})\text{Me}_2$ ]

AIDS = Acquired immunodeficiency syndrome

Ala = Alanine

Aliquat 336 = tricaprylmethylammonium chloride

AMV = Avian myeloblastosis virus

Arg = Arginine

Asn = Asparagine

Asp = Aspartic acid

AZT = 3'-azido-2',3'-dideoxythymidine

BP = Binding pocket

CAN = Ceric ammonium nitrate

cDNA = Complementary DNA

CI = Chemical ionization

Cys = Cysteine

DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene

DDDP = DNA-dependent DNA polymerase

ddC = 2',3'-dideoxycytidine

ddI = 2',3'-dideoxyinosine

DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

dG = 3'-deoxyguanosine

DMF = dimethylformamide

DMSO =dimethyl sulfoxide

DNA = Deoxyribonucleic acid

dNTP = Deoxynucleoside triphosphate

ds = Double stranded

dT = 3'-deoxythymidine

dTTP = Deoxythymidine triphosphate

EIMS = Electron impact mass spectrometry

FDA = Federal Drug Administration

FPV = Flash vacuum pyrolysis

Gln = Glutamine

Glu = Glutamic acid

His= Histidine

HIV-1 = Human immunodeficiency virus type 1

HIV-2 = Human immunodeficiency virus type 2

HOMO = highest occupied molecular orbital

HPLC = High performance liquid chromatography

HMPA =hexamethylphosphoramide

HRMS = High resolution mass spectrometry

Hz = Hertz

IC<sub>50</sub> = Inhibition concentration where 50% of the enzyme activity is inhibited

Ile = Isoleucine

IN = Integrase

kb = Kilo base

LAH = Lithium aluminum hydride

Leu = Leucine

LUMO = Lowest unoccupied molecular orbital

Lys = Lysine

Met = Methionine

mRNA = Messenger RNA

m/z = Mass to charge ratio

NBS = *N*-bromosuccinimide

NMR = Nuclear magnetic resonance

NNI = Non-nucleoside inhibitor

NNIBP = Non-nucleoside inhibitor binding pocket

NNIBS = Non-nucleoside inhibitor binding site

nOe = Nuclear Overhauser effect

PBS = Primer binding site

PCC = Pyridinium chlorochromate

Phe = Phenylalanine

PM3 = Reparameterization of AM1 (Austin Method 1)

PPA = Polyphosphoric acid

PR = Protease

Pro = Proline

psi. = pounds per square inch

rA = Adenosine

rC = Cytidine

RDDP = RNA-dependent DNA polymerase

RNA = Ribonucleic acid

RNase H = Ribonuclease type H

RT = Reverse transcriptase

Ser = Serine

sp. = Species

ss = Single stranded

STN = Streptonigrin

TCA = Trichloroacetic acid

THF = Tetrahydrofuran

Thr = Threonine

TIBO = Tetrahydroimidazobenzodiazepinethione

TLC = Thin layer chromatography

tRNA = Transfer RNA

Trp = Tryptophan

Tyr = Tyrosine

UC 84 = Uniroyal Compound 84

Val = Valine

## Chapter 1: Introduction

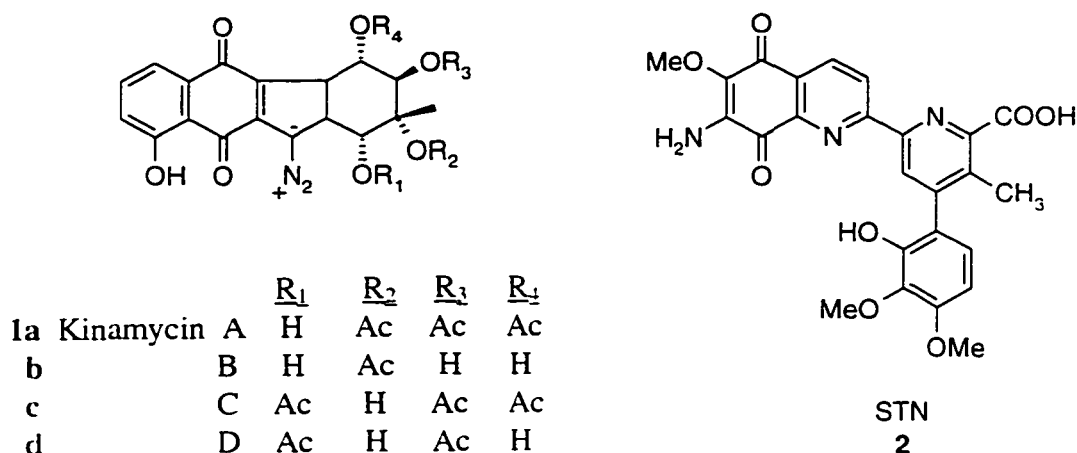
### 1.1 Naturally Occurring Quinones

Naturally occurring quinones have captured human attention for thousands of years, initially by reason of their bright colors with possible uses as dyes, and as drugs.<sup>1</sup> Pigments of various colors, now characterized as quinones, have been isolated from high and lower plants, fungi, as well as from animals.<sup>2</sup> Crude preparations of plants, presently known to contain quinones as active ingredients, were prescribed more than 4000 years ago as purgatives or drugs.<sup>1,2</sup> Throughout history, several other medicinal benefits have been adding on to the list associated with the use of naturally occurring quinones.<sup>1</sup>

The discoveries of antibiotic and antitumor properties assigned to several naturally occurring quinones have raised interest among scientists for use as pharmaceuticals. Some natural quinones, such as the kinamycins A, B, C, and D **1a-d**<sup>3,4,5</sup> and streptonigrin (STN) **2**<sup>6</sup>, are only weak antibiotic antitumor agents but have attracted attention because of novel structural features which suggest unusual biosynthetic processes and possibly novel mechanisms of bioactivity.

Recently, during the screening of natural products for inhibition against the HIV virus, STN has demonstrated one more interesting facet by testing positive as an inhibitor toward the reverse transcriptase (RT) of HIV-1.<sup>7</sup> This revelation, along with other observations of simpler quinones demonstrating anti-RT activity, implies that quinone analogs might also offer promising results in the fight against AIDS.<sup>8</sup>

Figure 1

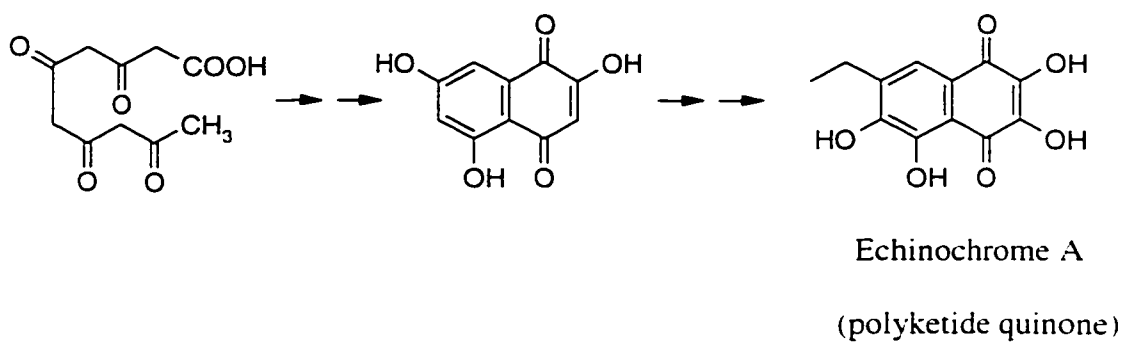


### 1.1.1 Biosynthesis of *p*-Quinones

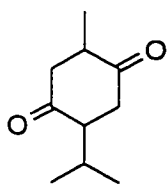
The biosynthesis of the skeleton of naturally occurring quinones can originate from different carbon sources. Acetate and isoprene units are the two major building blocks used in the *de novo* synthesis of quinone skeletons.<sup>9</sup> The term polyketide quinone and terpenoid quinone refer respectively to quinones possessing a skeleton originating from the acetate/malonate pathway (Scheme 1) or the isoprenoid pathway.<sup>9</sup> Depending on the number of isoprene units present in the isoprenoid quinone, the term monoterpene (2 units or 10 C), sesquiterpene (3 units or 15 C), and diterpene (4 units or 20 C) is employed (Figure 2).<sup>9,10</sup> The diterpene skeleton is the most abundant and accounts for about two-thirds of the hundred or so known naturally occurring terpenoid quinones.<sup>9</sup> When derivatives of isoprene units are present only in the side chains of quinones, the term meroterpene is utilized.

Vitamin K and the coenzyme Q are examples of biologically important meroterpenoids (Figure 3).<sup>9</sup>

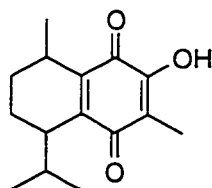
### Scheme 1 Quinones of acetate / malonate origin



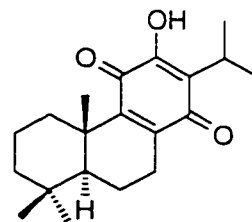
### Figure 2 Quinones of isoprenoid origin



Thymoquinone  
(monoterpenoid)

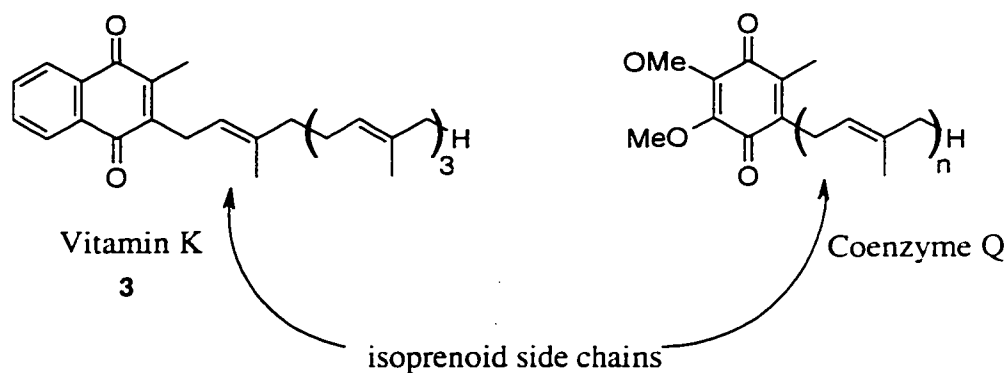


Mansonone B  
(sesquiterpenoid)



Royleanone  
(diterpenoid)

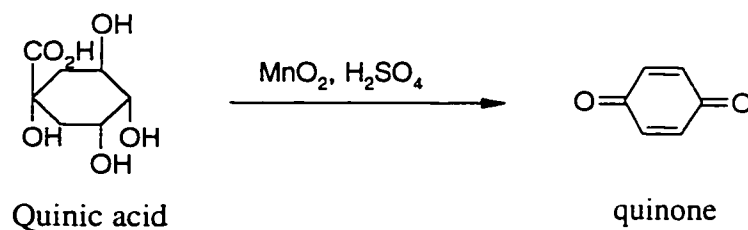
**Figure 3 Meroterpenoids**



### 1.1.2 Chemical Synthesis of Quinones

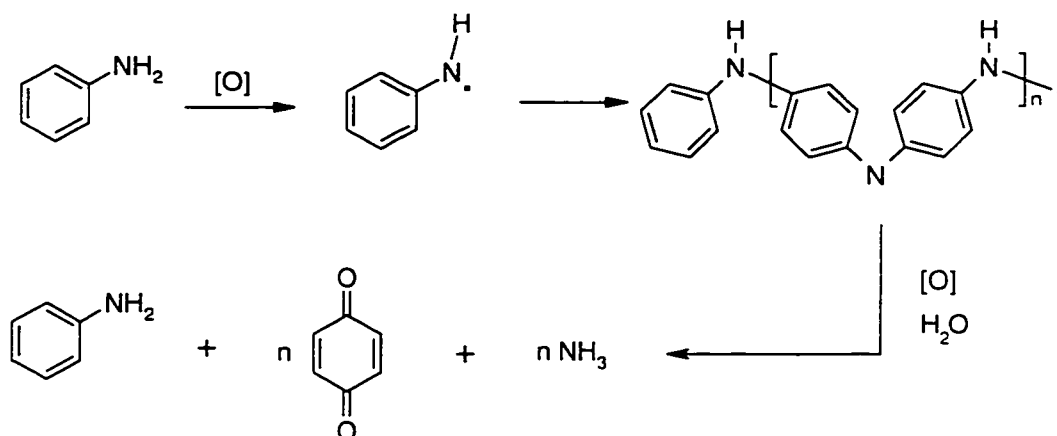
Since the mid 19<sup>th</sup> century, chemists have been studying the chemical properties of various quinones. The first-synthesized and most common quinone, *p*-benzoquinone, was discovered in the late 1830's in Liebig's laboratory as the result of the oxidation of quinic acid with manganese dioxide and sulfuric acid. This reaction involves a dehydration, decarboxylation, and oxidation (Scheme 2).<sup>2</sup>

**Scheme 2**



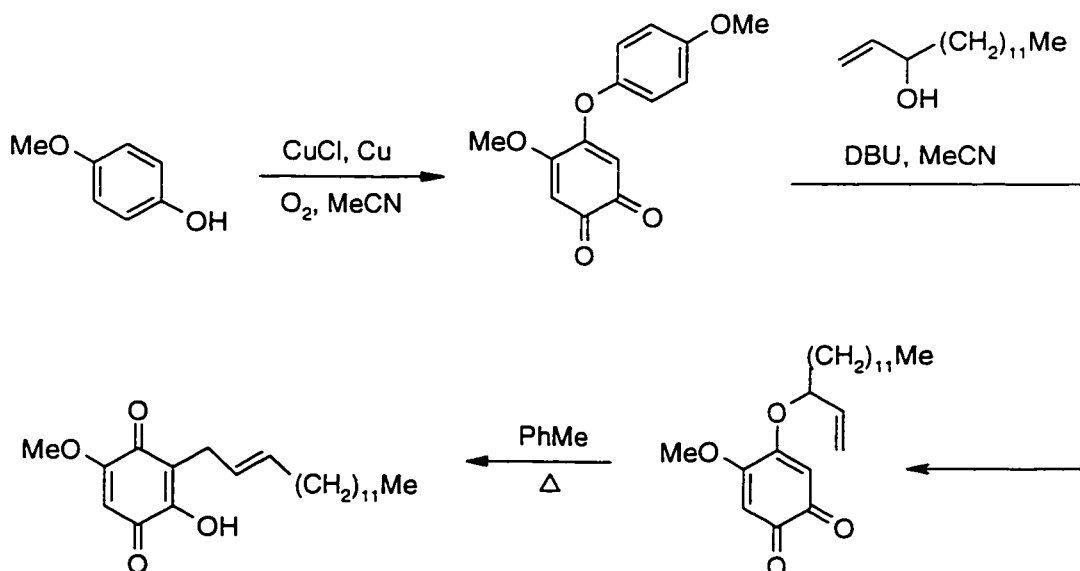
These same reagents can also react with aniline via a free radical condensation mechanism to afford the same end product, *p*-benzoquinone (Scheme 3).<sup>2</sup>

Scheme 3



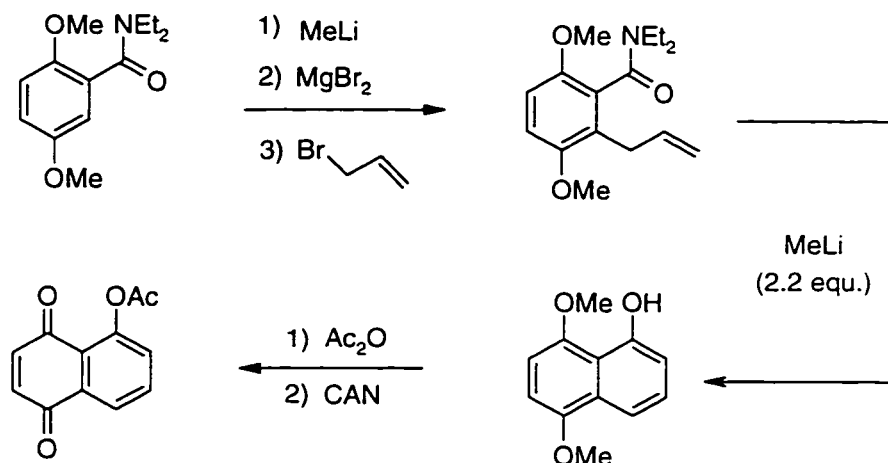
Biologically active unsymmetrical alkylhydroxymethoxyquinone analogs can be synthesized from *p*-methoxyphenol. The alkyl side chain can be introduced regiospecifically *ortho* to the hydroxy group via a Claisen rearrangement (Scheme 4).<sup>11</sup>

Scheme 4



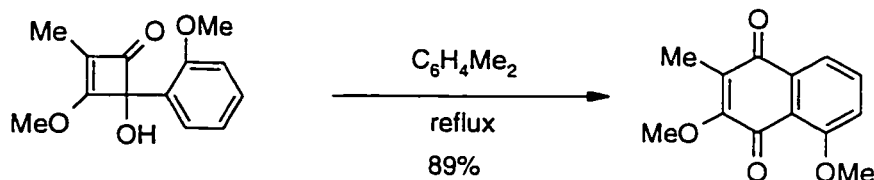
Several methods of synthesizing naphthoquinones having 1 to 6 substituents can be found in the literature.<sup>9</sup> For example, the regiospecific synthesis of several mono- and dimethoxynaphthol intermediates have afforded the corresponding quinones in yields of 50-60% (Scheme 5).<sup>12</sup>

Scheme 5



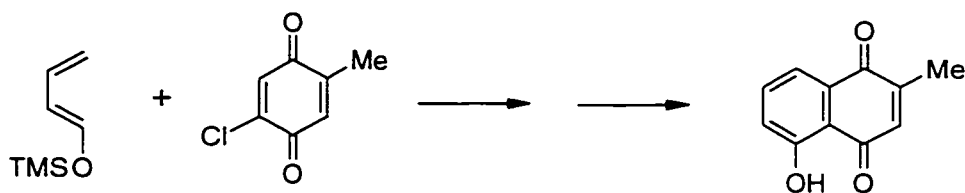
Another interesting method involves the rearrangement of hydrocyclobutenone derivatives (Scheme 6).<sup>13</sup>

Scheme 6



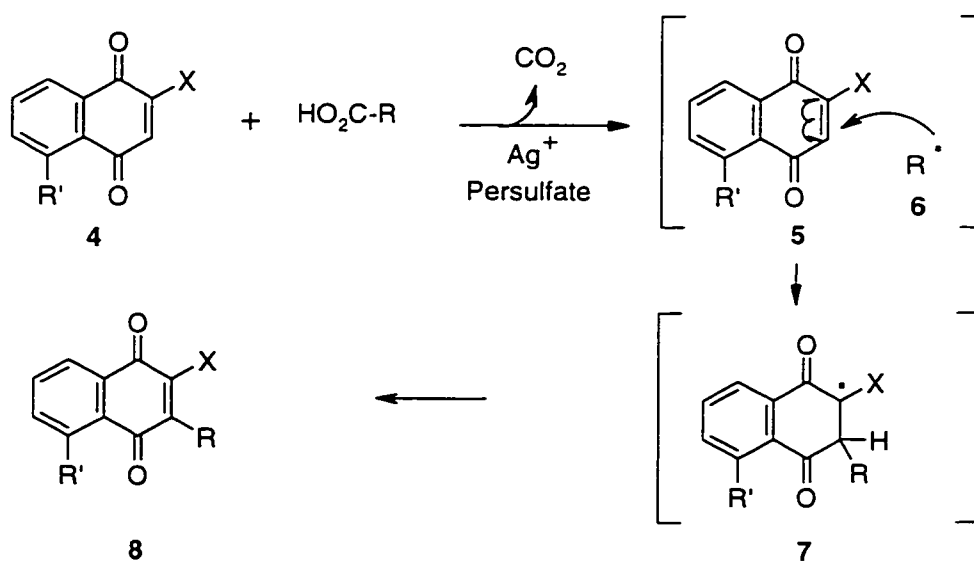
Diels-Alder reactions can also be utilized in order to add the necessary new ring to the skeleton of benzoquinones to give the corresponding naphthoquinones (Scheme 7).<sup>14</sup>

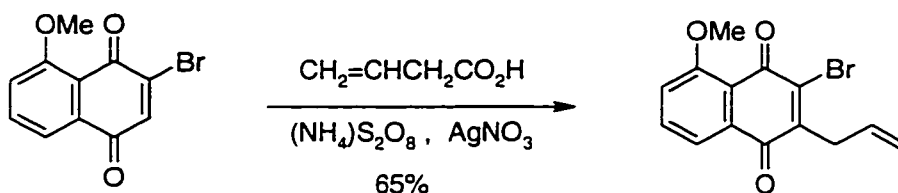
## Scheme 7



Alkylation at the 2 and 3 position of 1,4-naphthoquinones is possible via the Jacobsen-Thorsell oxidative coupling method (Scheme 8).<sup>15</sup> In this process, a carboxylic acid ( $\text{RCO}_2\text{H}$ ), incorporating the group R to be added to the quinone ring system, is treated with ammonium persulfate in the presence of a catalytic amount of  $\text{Ag}^+$  and the quinone substrate **4**. Oxidative decarboxylation ensues and results in the generation of a free radical ( $\text{R}\cdot$ ) **6** which adds to the quinone to yield an intermediate radical species **7**. Further oxidation of **7** *in situ* leads to the desired end product **8**.

## Scheme 8





### 1.1.3 Chemistry of Quinones

The term “quinone” refers generally to a 1,4-diketone formally derived from dihydro aromatic compounds in which the two carbonyl groups are connected by a system of conjugated double bonds.<sup>10</sup> The reversible oxidation-reduction reactions of biologically active quinones plays a key role in several biological processes. One example is found in the biological activity of vitamin K (3). The oxidation of the reduced form of Vitamin K is of major importance in blood coagulation since a deficiency of this vitamin leads to hemorrhagic bleeding.<sup>10</sup> The reduced form of vitamin K (9) reacts, via the enzyme 10, with oxygen and carbon dioxide to generate an interesting 2,3-epoxide intermediate 11 during this process (Scheme 9).<sup>16</sup>

Several synthetic quinones like diphenoquinone 12 ( $E_0^{\text{alc.}} 0.954 \text{ V.}$ ) are powerful oxidizing agents.<sup>2</sup> Quinones such as 1,4-benzoquinone 13, chloranil 14, and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) 15 (Figure 4) are widely use in synthetic organic chemistry as oxidants or dehydrogenating agents (Scheme 10).<sup>1</sup>

Scheme 9

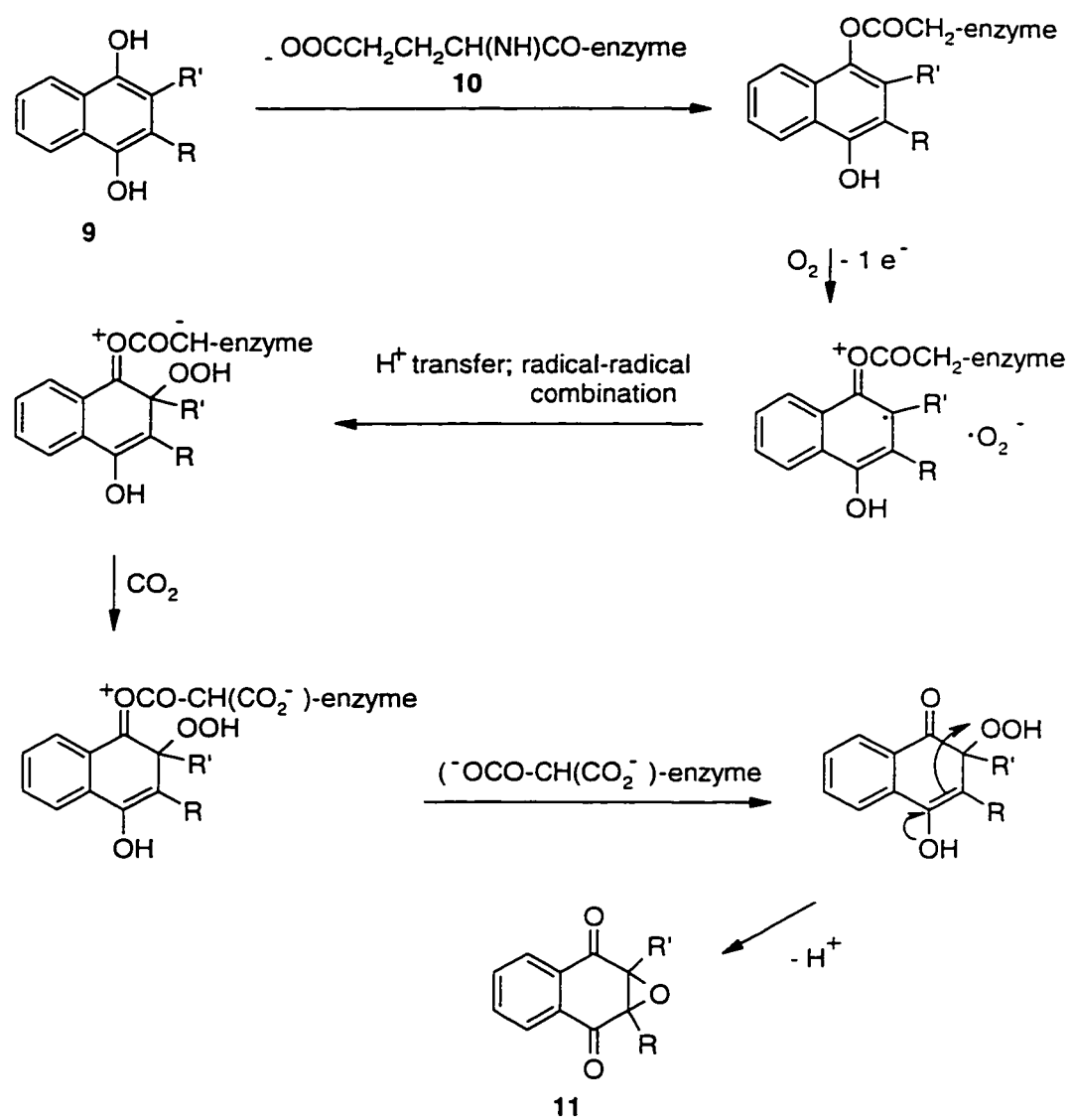
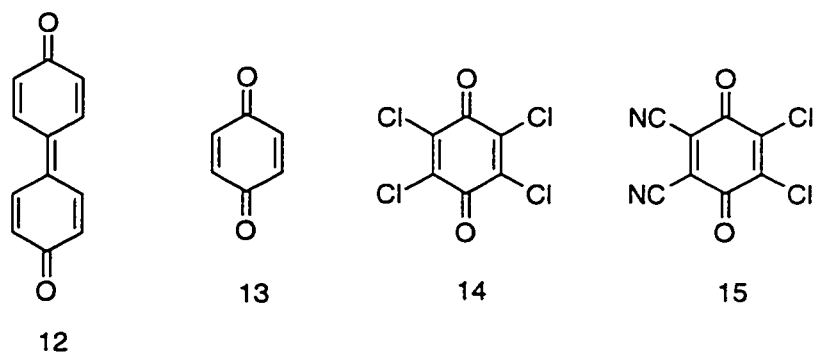
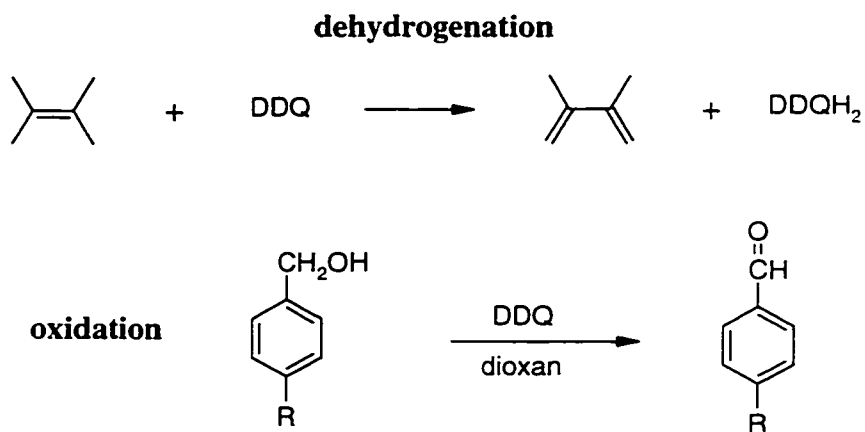


Figure 4

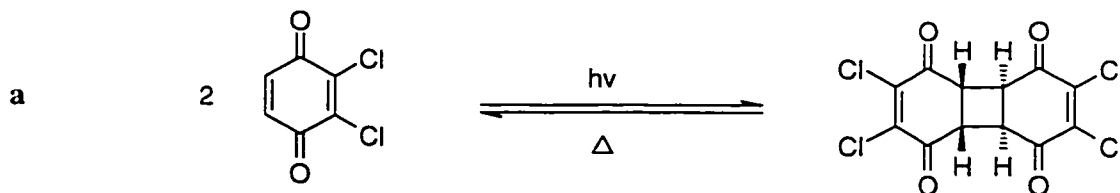


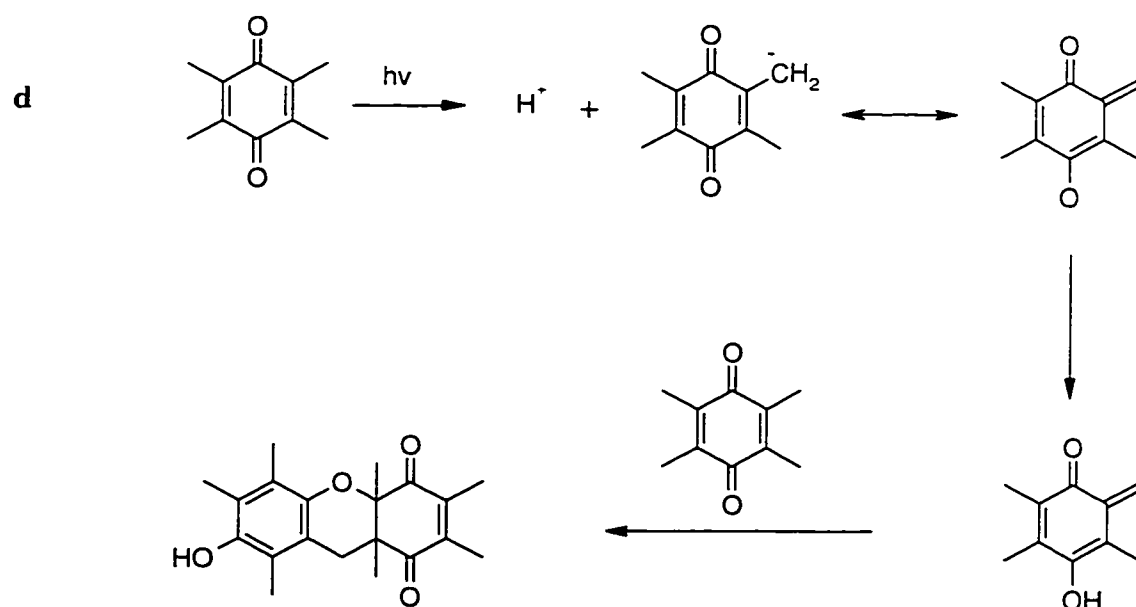
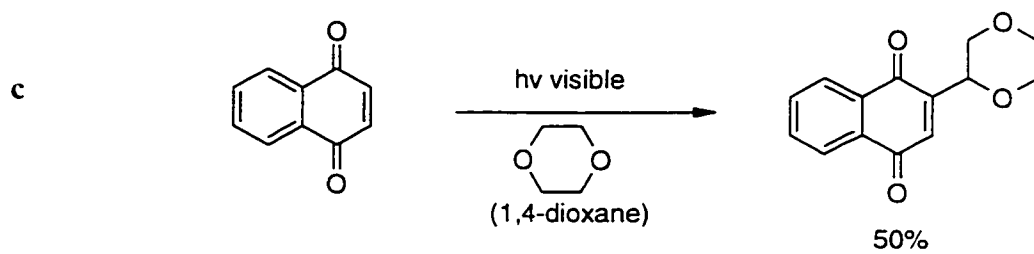
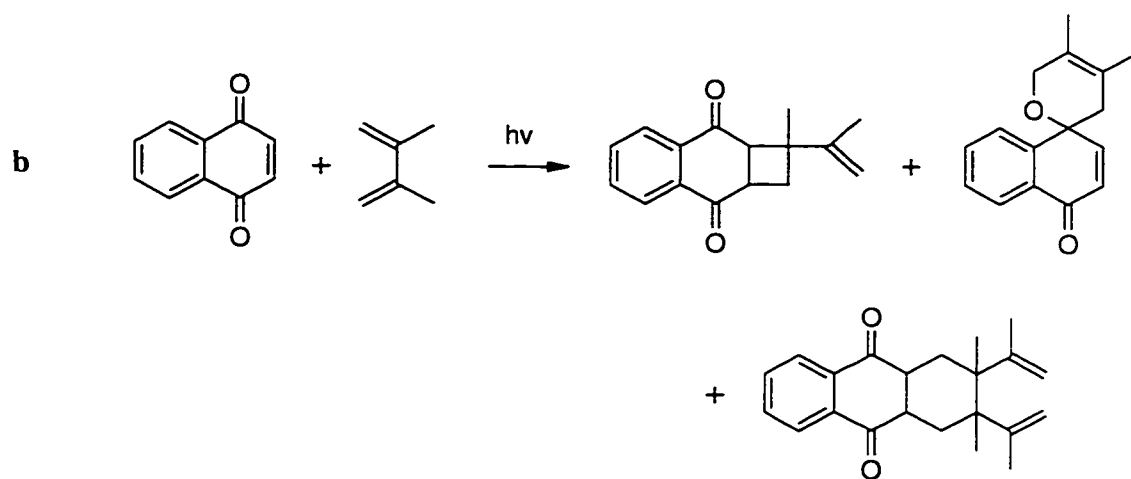
Scheme 10

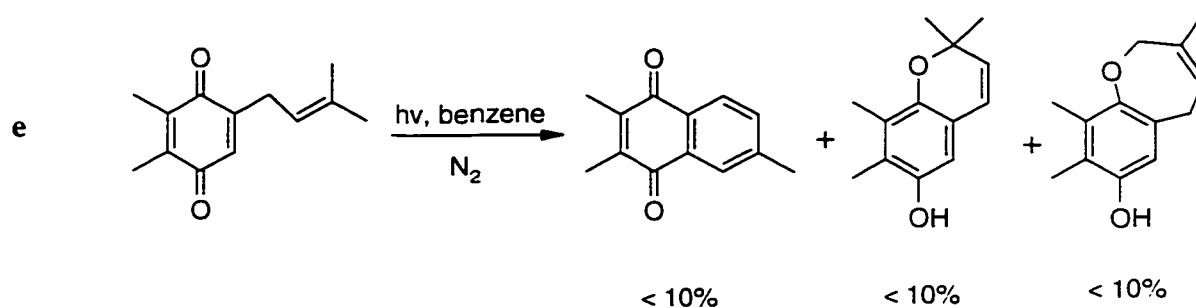


Since quinones readily undergo light-induced reaction, manipulation and storage of these compounds should be made accordingly.<sup>1</sup> Interestingly, the ingestion by animals of the red fluorescent quinone pigment of plants from the genus *Hypericum* causes them to become light sensitive.<sup>1</sup> Photochemical experiments with various quinones are well documented. They can react either intermolecularly with another quinone (Scheme 11a), an olefin (b), or a hydrogen-donor (c), or intramolecularly with an appropriate part of an attached side-chain varying from the methyl groups (d) of duroquinone to the polyisoprenoid systems found in naturally occurring quinones (e).<sup>1</sup>

Scheme 11

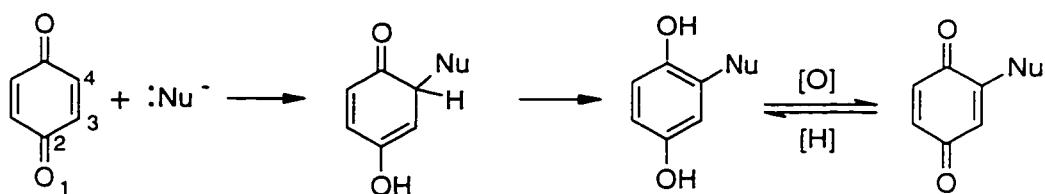




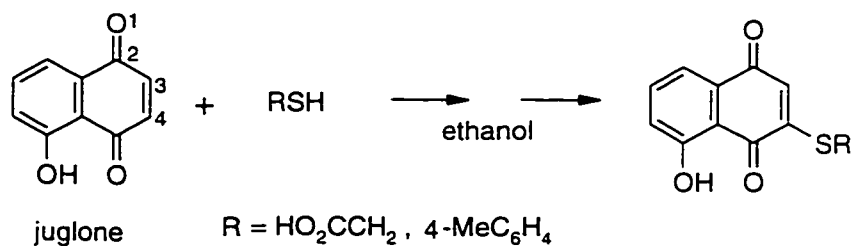


The chemistry of quinones is similar to that of open-chain  $\alpha$ ,  $\beta$ -unsaturated ketones. They are, however, more reactive than the latter and can undergo a wide range of 1,4-reductive additions of the Michael type (Scheme 12 and 13).<sup>1,2</sup>

Scheme 12

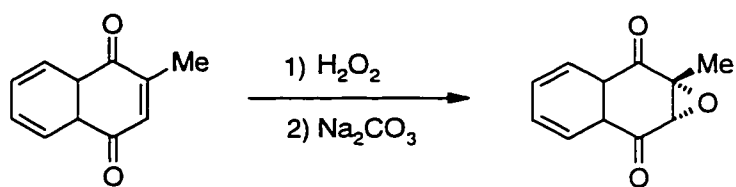


Scheme 13

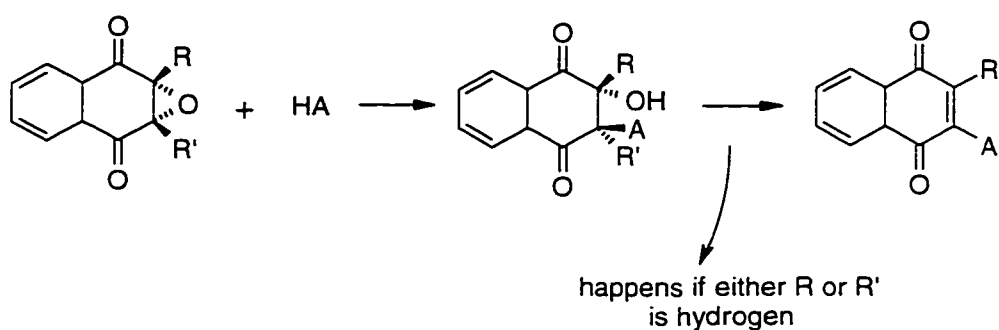




Scheme 16



Scheme 17



In this laboratory, interest in *p*-quinones is related to two areas of research. The first one involves the exploration of inhibitors of the reverse transcriptase of HIV-1, the causative agent for AIDS. The second concerns certain polyketide derived quinones and hydroquinones related to the antitumor antibacterial compounds called the kinamycins. The significance of *p*-quinones in each of these areas is outlined below.

## 1.2 Quinones as Inhibitors of the HIV-1 RT

### 1.2.1 HIV Virus

About 15 years ago, AIDS (acquired immunodeficiency syndrome) research started all over the world as, for the first time, the retrovirus causing the break-down of the human immune system was isolated.<sup>17</sup> It is expected that by the year 2000, as many as 40 million people world-wide will be infected by this deadly retrovirus.<sup>18</sup> Already, AIDS is the first and fourth leading cause of death for men and women, respectively, in the 25-44 age group in the USA.<sup>18</sup>

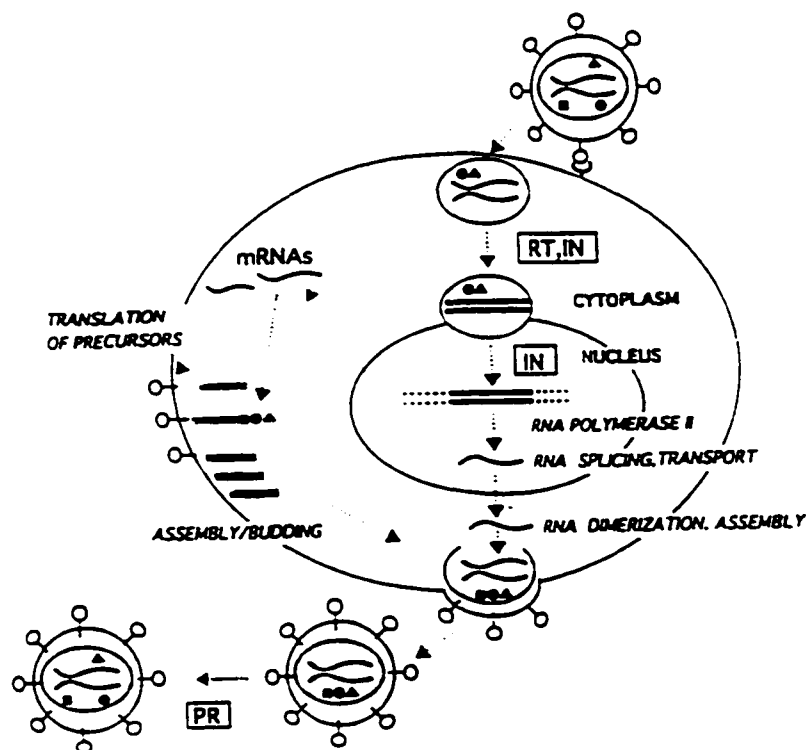
The retrovirus in question, the human immunodeficiency virus type 1 (HIV-1), just like any retrovirus, has the ability to insert DNA copies of its RNA viral genome into the chromosomes of a host cell.<sup>17</sup> In the case of HIV, the principal host cell is the T helper cell (a sub-class of white blood cells) which is essential for the human immunodefence system.<sup>19</sup> The HIV virus binds to this cell via a receptor (CD4) on the surface of the T helper cell.<sup>19</sup> The exact cause of cell death is complex and not well understood at this time.<sup>19</sup> Replication of the RNA genome requires that the genetic material of the virus be expressed by the host transcription system. As a result, the viral single stranded (ss) RNA must be converted into a double-stranded (ds) DNA intermediate in order to be inserted into the host genome.<sup>19</sup>

The crucial steps in the retroviral life cycle consist of the conversion of the viral RNA genome into proviral DNA,<sup>17</sup> the integration of this DNA into the host genome, and the transcription of the DNA provirus back to RNA either to form the genome of a new virion or

to be converted back to DNA and then integrated in the DNA chromosome of the present host cell.<sup>19</sup>

Three key viral enzymes (the protease (PR), the reverse transcriptase (RT) and the integrase (IN)) are used by the HIV virus in order to incorporate its RNA genome into the DNA of the host cell.<sup>19</sup> An extensive research effort is underway to find ways to prevent these enzymes from carrying out their catalytic activity. The role of the protease is to specifically cleave the long polypeptide precursors, obtained from the translation of the genome, into "mature" proteins. Reverse transcriptase is essential for converting the RNA genome of the virus into a ds DNA which will be inserted into the host chromosome by the integrase.<sup>19</sup>

**Figure 5 The life cycle of the HIV virus**



### 1.2.2 Reverse Transcriptase

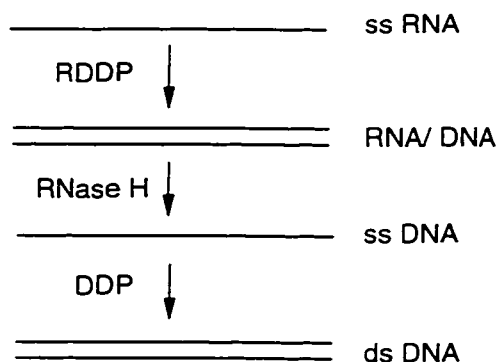
The first reverse transcriptase (RT) was discovered in 1970 in the laboratories of Temin and Baltimore as a component of RNA tumor viruses.<sup>20, 21</sup> The discovery of this enzyme was the first proof that genetic information could sometimes flow “backward” from RNA to DNA.<sup>22</sup>

As mentioned above, this essential viral enzyme (RT) plays a key role in the early stages of HIV infection.<sup>17, 19, 23</sup> It is, therefore, an attractive target for potential drug therapy especially since it is not required for normal host cell metabolism.<sup>17</sup> This multifunctional enzyme has an inherent ribonuclease (RNase H) activity and exhibits both RNA-dependent DNA polymerase (RDDP) and DNA-dependent DNA polymerase activities (DDDP) which all act in a concerted manner to convert the viral RNA into a ds DNA.<sup>19</sup>

Four major steps are needed for an effective conversion of genomic RNA to proviral DNA and are all accomplished by RT (Figure 6). The first step consists of copying the (+) RNA viral strand ( (+) because the viral RNA itself codes for the protein products) into a complementary (-) DNA strand. This enzymatic activity is carried out by the RDDP. The primer used in order for this synthesis to take place is obtained from the host cell and consists of human tRNA<sup>Lys3</sup>.<sup>24</sup> This primer anneals to an eighteen nucleotide long complementary RNA sequence located on the viral RNA strand (the primer binding site or PBS).<sup>17</sup> The second step involves the displacement of this newly synthesized DNA strand to the 3'-end of the same or second viral RNA molecule where completion of this minus strand is made.<sup>25</sup> Once the complete synthesis of the (-) DNA strand is accomplished, the original RNA

template must be removed by the RNase H in order to synthesize a new (+) DNA strand.<sup>17</sup> This effective and orderly degradation of the viral RNA template represents the third step. The newly synthesized (-) DNA strand can then serve as a template for the synthesis of the (+) strand DNA with the help of an RNase H- resistant RNA oligonucleotide (polypurine tract) as a primer.<sup>19</sup> This DNA synthesis is carried out by the DDDP activity of RT<sup>25</sup> and corresponds to the fourth and last step of the conversion. Once the double stranded proviral DNA molecule is obtained, it is then integrated into the nuclear DNA of the host cell by the HIV-1 integrase enzyme.<sup>17, 23</sup>

**Figure 6 Steps in the conversion of ss RNA into ds DNA**



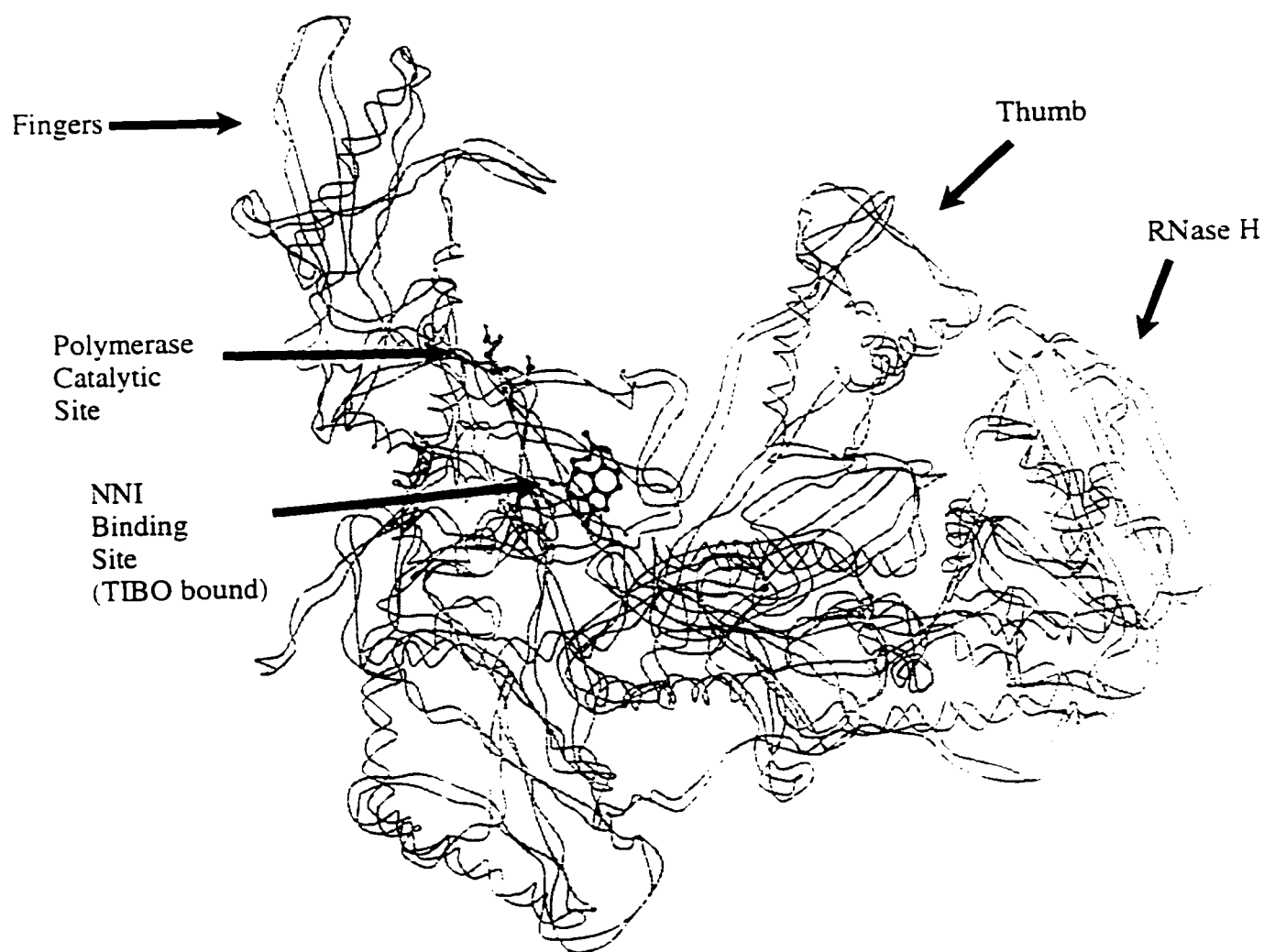
### 1.2.3 Structure of RT

HIV1-RT is an asymmetric heterodimer composed of two subunits with molecular weight of 66 kDa (p66) and 51 kDa (p51).<sup>17, 26</sup> The p51 polypeptide is identical in primary sequence to the p66 except that the carboxyl terminal (C-terminal) portion containing the RNase H activity has been proteolytically cleaved (between position 440 and 441 of the

amino acid sequence) by HIV-1 protease.<sup>27, 28</sup> The DNA polymerase domain resides in the 51 kDa N-terminal portion of p66 while the RNase H domain is located in the remaining 15 kDa C-terminal portion.<sup>17</sup>

All enzymatic activity (DNA polymerase and RNase H) resides in the p66 subunit which is considered to be the catalytic core of the enzyme.<sup>17</sup> The role of the p51 subunit is still unclear but it is believed that it forms a portion of the template-primer binding site.<sup>26, 29</sup> The crystal structure of HIV-1 RT at 3.5 Å shows that even though the polymerase domains of both subunits have the same sequence, the conformations are very different.<sup>26</sup> The residues thought to be involved in the catalytic activity of the polymerase (Asp 185, Asp 186, Asp 110) are essentially buried in p51 due to an alteration of the structure following the removal of the C-terminal portion mentioned above.<sup>26</sup> This would explain why only the p66 subunit has enzymatic activity.<sup>26</sup> The three residues mentioned above (Asp 185, Asp 186, Asp 110) are present in the palm subdomain of p66 and are highly conserved in polymerases.<sup>29</sup> These residues most likely coordinate the required catalytic metal ion and probably participate in nucleoside binding.<sup>17, 29</sup>

**Figure 7 Representation of RT with a Bound Inhibitor (TIBO). The Different Subdomains are Shown and the Cleft is Visible. (Generated by using Sybyl 6.3 ® based on X-ray crystallographic data deposited in the Brookhaven Protein Data Bank as entry 1hmv**



## 1.2.4 Inhibitors

There are two classes of RT inhibitors: nucleoside analogues and non-nucleoside inhibitors (NNI's).

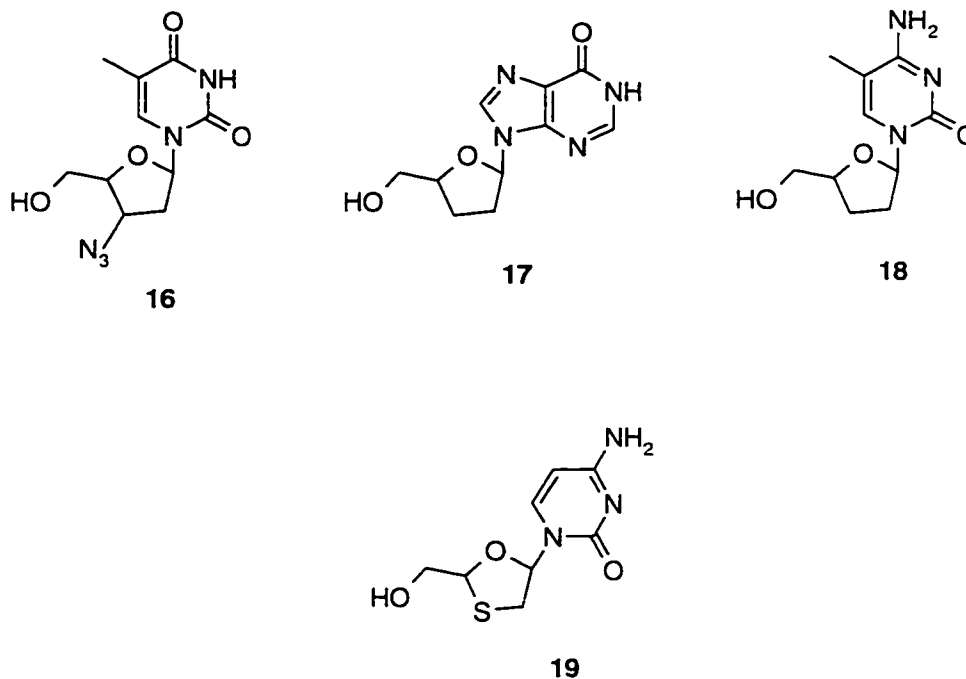
### 1.2.4.1 Nucleoside analogues

Nucleoside analogues (or nucleoside inhibitors, chain terminators, or competitive inhibitors) are synthetic nucleosides which lack the 3'-OH on the sugar ring of the nucleotide essential for elongation of the DNA strand.<sup>23</sup> Termination of the DNA chain occurs and the virus cannot replicate. The precise mechanism of nucleotide addition is still uncertain.<sup>19</sup>

Portions of the fingers subdomain serve to bind the ss RNA of the template. A protein conformational change is observed during nucleotide incorporation. This change in conformation is necessary in order to position the incoming nucleotide for nucleophilic attack. It is believed that the nucleophilic attack, by the oxygen atom of the 3'-OH group of the growing DNA strand, could be metal-mediated.<sup>19</sup>

The principal AIDS drugs of this class are AZT (3'-azido-2',3'-dideoxythymidine) **16**, ddI (2',3'dideoxyinosine) **17**, and ddC (2',3'dideoxycytidine) **18** (Figure 8).<sup>23</sup> The effectiveness of these inhibitors is limited by the rapid emergence of resistant viral strains caused by mutations and by their lack of specificity for the viral RT since they can also inhibit normal cellular DNA polymerases.<sup>23, 30</sup>

Figure 8



Recently, an interesting nucleoside analogue, 3TC (2',3'-dideoxy-3'-thiacytidine) 19 has achieved FDA approval.<sup>31</sup> Although mutation of RT still occurs with 3TC, HIV-1 RT remains sensitive to other inhibitors such as AZT and is less prone to further mutations. Thus, combination of 3TC with other nucleoside inhibitors may help to decrease the resistance problem.<sup>31</sup>

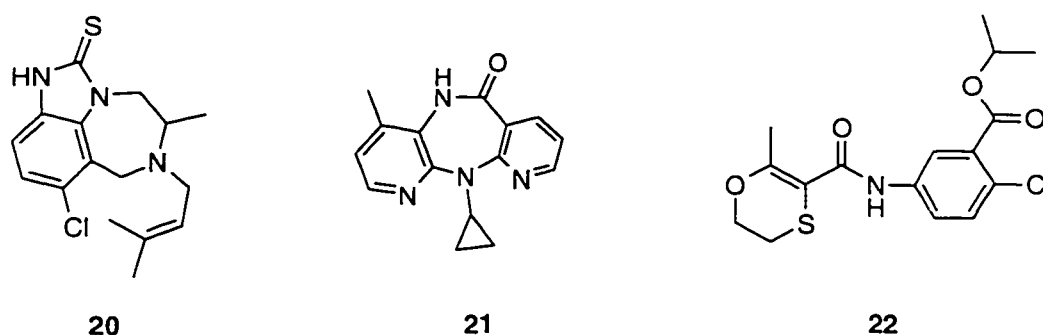
#### 1.2.4.2 Non-nucleoside inhibitors

Unlike the nucleoside analogues, the non-nucleoside inhibitors are highly specific and selective regarding HIV-1 RT and are, therefore, relatively non toxic to human cells and HIV-infected patients.<sup>32, 33</sup> These compounds show no inhibitory activity against human DNA

polymerases  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ , or other RT's present in HIV-2 or avian myeloblastosis virus (AMV).<sup>19</sup> The recent discovery of this class of compounds resulted from widespread screening of synthetic and natural molecules for anti-HIV activity.<sup>17, 30, 34</sup>

Some examples of this class are TIBO<sup>23</sup> (tetrahydroimidazobenzodiazepinone) **20**, nevirapine<sup>23</sup> (a dipyridodiazepinone derivative) **21**, and UC 84<sup>35</sup> **22** (Figure 9).

**Figure 9**



These inhibitors do not bind at the dNTP-binding site and act by allosteric inhibition. Non-nucleosides are found to be non-competitive inhibitors with respect to incoming nucleotides.<sup>34</sup> Unlike the nucleoside inhibitors, their mechanism of action is not yet understood in detail.<sup>17</sup>

Even though these inhibitors are strikingly different from a structural point of view and fall into several chemical groups, they all have in common an aromatic moiety which enables them to bind to the same hydrophobic pocket<sup>23</sup> and they may very well share the same basic inhibition mechanism.<sup>19</sup>

The non-nucleoside inhibitor binding pocket (NNIBP), where all non nucleotide inhibitors (NNI's) appear to bind, is located between the palm and the base of the thumb subdomains close to the expected site of binding of the 3' terminus of the primer. but not overlapping the anticipated DNA binding sites.<sup>26</sup>

This deep pocket, like the NNI, is mostly hydrophobic with substantial aromatic character (Tyr 181, Tyr 188, Phe 227, Trp 229, and Tyr 232).<sup>23</sup> The Van der Waals interactions between the side chains of the pocket and the aromatic moiety of the inhibitor seems to drive the binding and could well explain the fact that structurally different inhibitors are possible.<sup>23, 36</sup>

A few hydrophilic residues (Lys 101, Lys 103, Ser 105, Glu 138) are also seen at one end of this pocket, the so-called "entrance", and it is possible that these residues and backbone atoms may form hydrogen bonds to some NNI.<sup>23</sup> X-ray crystallographic analysis, however, reveals that neither nevirapine<sup>36</sup> nor TIBO<sup>37</sup> (Figure 11) interact with the polar or charged side chains of these hydrophilic residues. Nonetheless, molecular modeling studies performed in this laboratory on recently discovered NNI's, such as indole **23**, suggest that H-bonding to other side chains, such as the carboxylate group of Glu 138 and the side chain ammonium group of Lys 103, is possible.<sup>38</sup> Val 106 is also believed to participate directly in the binding of NNI based on drug resistance studies.<sup>36</sup> It has been suggested that due to a very versatile architecture of the NNIBP, different NNI's bind in slightly different ways.<sup>23, 36</sup>

Figure 10

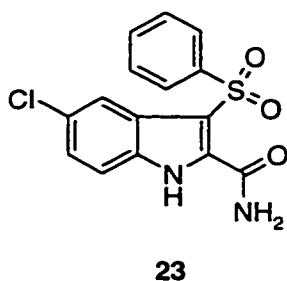
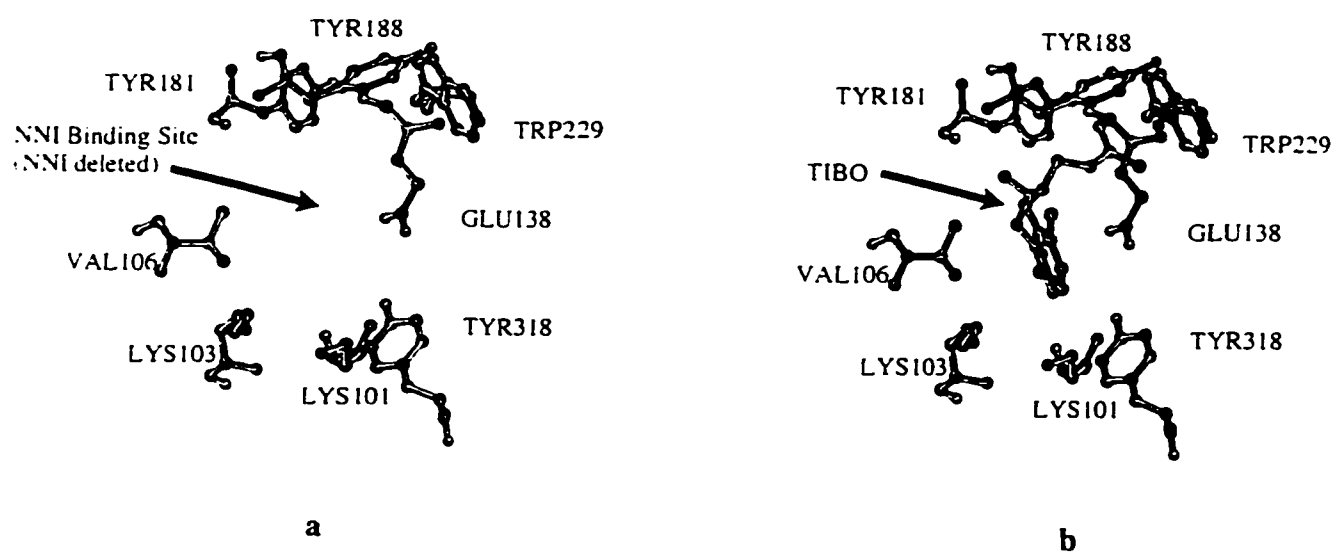


Figure 11 View of the NNIBP showing (a) the residues involved and (b) the NNIBP in the presence of the inhibitor TIBO.



Upon binding of the NNI, the NNIBP changes conformation in order to accept the NNI with consequent exposure of some residues in the pocket. For example, rotations of the

*p*-hydroxybenzyl side chain of Tyr 181 and Tyr 188 occur in order to enlarge the cavity to accommodate the NNI.<sup>23</sup>

There are several theories concerning how the NNI impair the DNA polymerase catalytic activities of RT. Among them is that the inhibitor may interact with the “floor” and/or the “roof” of the NNI binding pocket which is composed of residues involved in drug-resistance mutations.<sup>23</sup> This would affect the catalytic geometry and the mobility of the polymerase domain. It could also alter the crucial binding of the primer strand.<sup>23</sup>

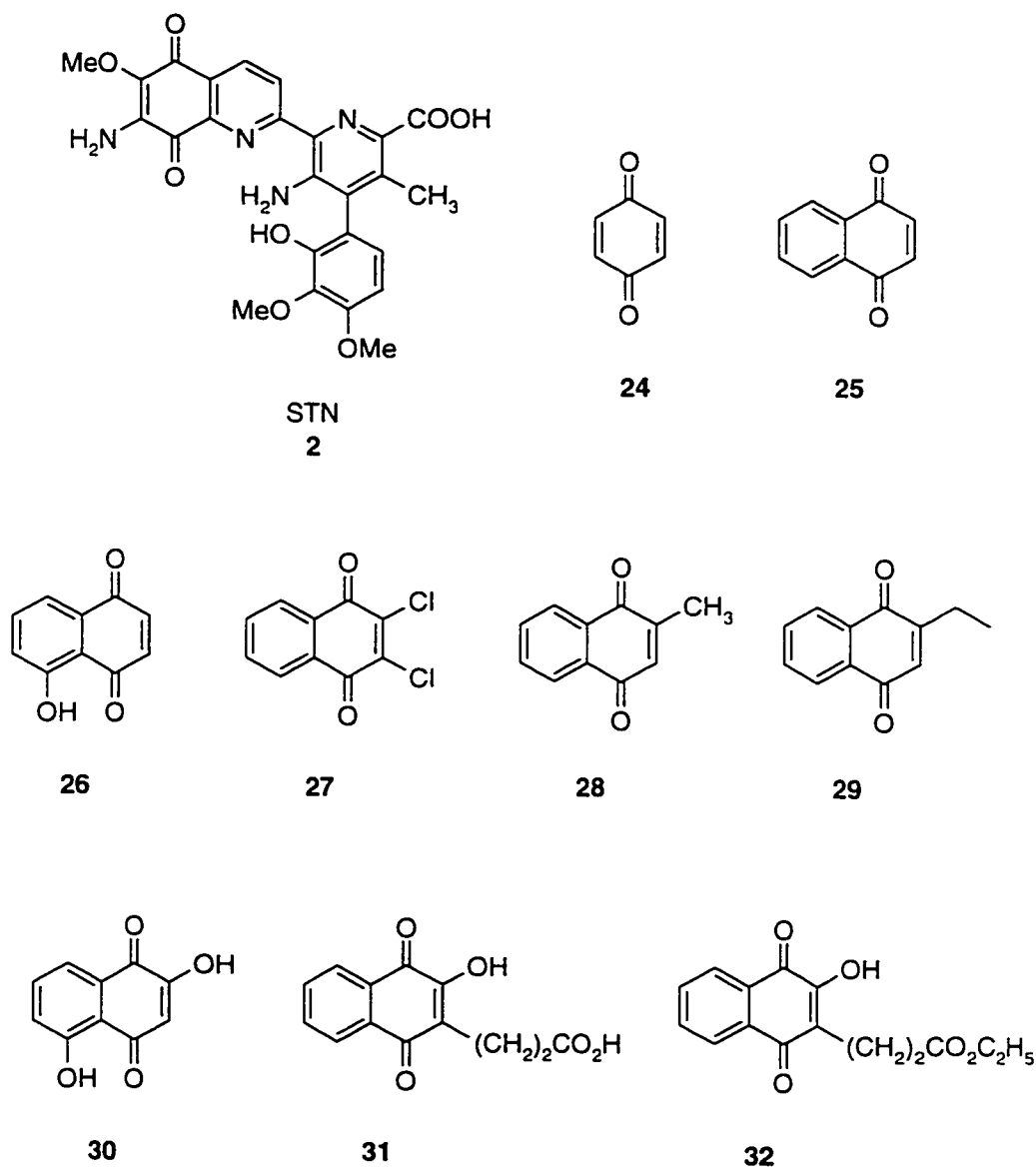
A persistent problem with the NNI class of anti HIV agents is the rapid development of resistant viral strains which have undergone point mutations in the NNIBP. As a result, there is a continuing interest in the discovery of novel NNI's which are less prone to the resistance problem.

### **1.2.5 Biologically Active *p*-Quinones**

Streptonigrin (STN) **2**, which was known to be an antitumor antibiotic,<sup>6</sup> was first shown to possess activity against RT by Chirigos et al.<sup>39</sup> who studied RT from AMV. Later, Hafuri et al.<sup>7</sup> demonstrated that inhibition of AMV RT was not the result of redox reactions of the quinone portion of STN and that other much simpler synthetic *p*-quinones could inhibit AMV RT. The term “quinone pocket” was used to refer to what was believe to be a common binding site on AMV RT which accommodated the quinone containing RT inhibitors. Later, a comparative study of the effectiveness of such quinones against mammalian DNA polymerases ( $\alpha$  and  $\beta$ ), HIV RT and AMV RT was reported.<sup>8</sup> From that study, several

naphthoquinones **25- 28** (Figure 12) were identified as structures with significant affinity for the HIV-1 RT relative to the mammalian DNA polymerases. Some of the structure-activity relationships observed in that study are summarized in Table 1.

**Figure 12**



**Table 1 Structure Activity Relationship of Quinone Derivatives**

Inhibition of RDDP activity (%)	
Drug concentration in $\mu\text{g/mL}$	HIV-1 RT (Poly (rA)/ oligo (dT))
Streptonigrin <b>2</b>	87
1,4-Benzoquinone <b>24</b>	15
<b>25</b>	92
<b>26</b>	95
<b>27</b>	85
<b>28</b>	70
<b>29</b>	0
<b>30</b>	0
<b>31</b>	0
<b>32</b>	0

The fact that these compounds possess significant activity against AMV RT as well as against HIV-1 RT is suggestive of a mode of binding to HIV-1 RT distinct from that of the other non-nucleoside inhibitors since that NNI's in general do not inhibit AMV RT. Thus, exploration of the nature of the "quinone-binding" site may eventually yield new class of anti HIV agents.

More recently, Loya et al.<sup>30</sup> have reported that 3,5,8-trihydroxy-4-quinolone, **33**, a natural product from the Red Sea sponge *Verongia* sp., and its dimethyl ether, **34**, are inhibitors of the RDDP activity of both HIV-1 and HIV-2, a second strain of HIV which is more prevalent in Africa.<sup>18</sup> It has been indicated by Bakhanashvili that the



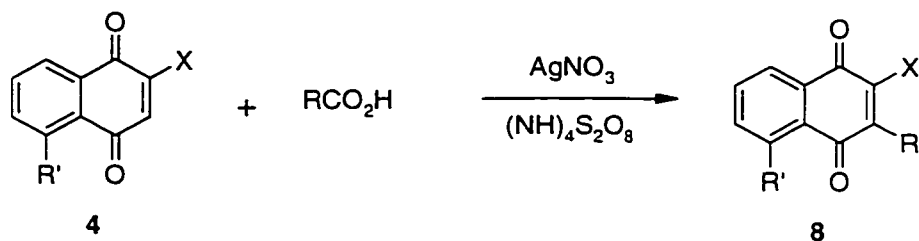
overall geometry of the pocket causing this resistance to inhibition in HIV-2, is however, deeper than this, since mutation of these two residues in HIV-2 to two tyrosines did not render HIV-2 sensitive to nevirapine.<sup>36</sup> In the case of the quinolones **33** and **34**, binding to RT may occur at a site completely distinct from that defined for the NNI for HIV-1 RT. Alternatively, binding of the quinolones may occur in the NNI binding site region but in a manner significantly different from that described for the other NNI's; that is, in such a way that the amino acid composition of the NNI site of HIV-1 and HIV-2 RT does not affect the affinity for the substantially different RT's. In the case of the quinones **24-32**, no data is available concerning the interaction with HIV-2 RT.

The present work was inspired by the possibility that the quinones and/or the quinolones might bind to a site on RT distinct from the NNI site. Such compounds would be expected to be effective against mutant strains of the HIV-1 RT which are resistant to the other known NNI inhibitors of RT and might be used therapeutically in combination with known NNI's. Whether or not the quinone binding site and the quinolone binding site are the same is unknown. In this study structural analogues of the quinones **25-32** were prepared and evaluated for effectiveness against HIV-1 RT.

### **1.2.6 Proposed Synthesis of *p*-Quinones Against RT**

The synthesis of analogues of the quinones was pursued in this work based on a free radical oxidative substitution reaction first developed by Jacobsen and Thorssell (Scheme 18; see Scheme 8 for mechanism).<sup>15</sup>

### Scheme 18 Jacobsen-Thorssell Reaction



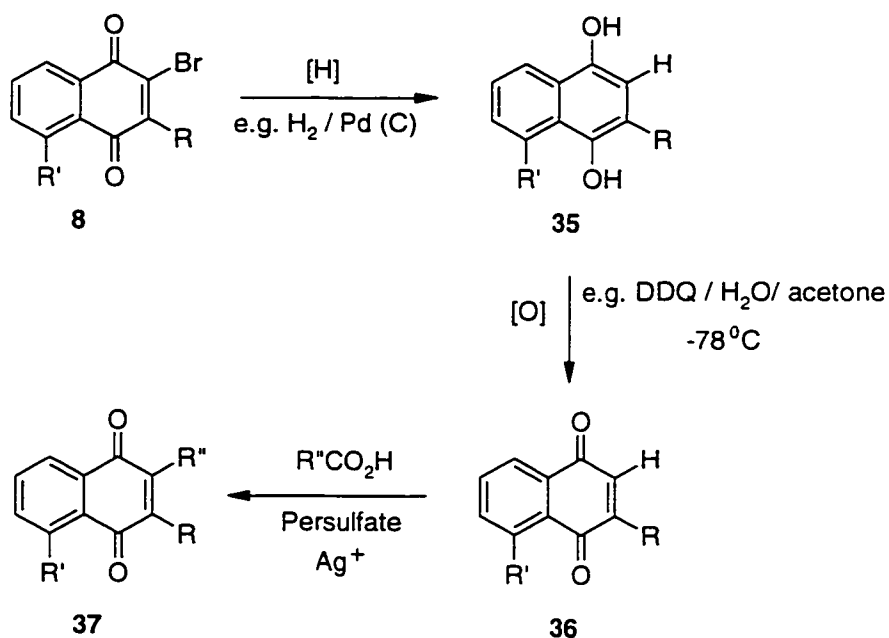
For quinones in which  $X \neq \text{H}$ , the addition occurs exclusively as shown such that the R group replaces the hydrogen atom on the quinone C=C bond. When this study began, it had been shown in this laboratory, that this process proceeds well for  $X = \text{Br}$  with  $\text{R} = \text{alkyl, benzyl, and aryl}$ . Thus, it was proposed that a variety of systems with  $X = \text{Br}$  would be prepared in this way. Furthermore, the brominated products **8** were intended to be employed as precursors to variously disubstituted quinones.

It was felt that the dehalogenation of the bromoquinone **8** might be performed reductively as shown below to yield the hydroquinone **35**. Oxidation of **35** to the quinone **36** would set the stage for an oxidation radical-mediated substitution using the  $\text{R}''\text{CO}_2\text{H}/$  persulfate/  $\text{Ag}^+$  method.

The use of brominated naphthoquinones as intermediates in the synthesis of the naphthoquinones of interest has the advantage that the methodology for regioselective

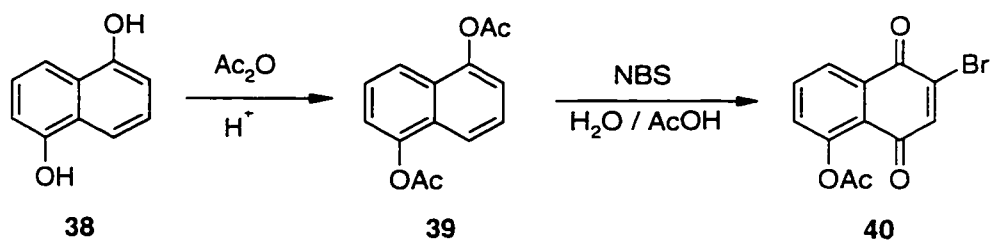
synthesis of bromonaphthoquinones bearing substituents in the aromatic ring is in place, at least for some substituents R'.

### Scheme 19 Synthesis of Naphthoquinones from Bromonaphthoquinones

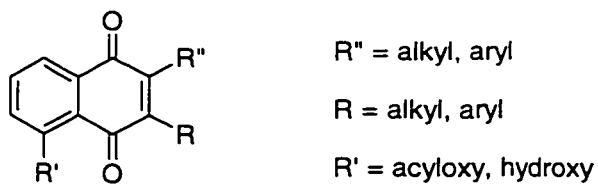


For example, 2-bromo-5-acetoxynaphthoquinone **40** can be prepared from readily available 1,5-dihydroxynaphthalene by reaction with NBS in aqueous acetic acid (Scheme 20). As a result, regiochemical control in the synthesis of naphthoquinones which are unsymmetrically substituted in the aromatic ring should be possible. Some examples of naphthoquinones which were intended to be synthesized to probe the dependence of anti-RT activity on naphthoquinone structure are shown below.

**Scheme 20 Synthesis of 2-Bromo-5-acetoxynaphthoquinone from  
1,5-Dihydroxynaphthalene**



**Figure 14 Examples of Naphthoquinones Serving as Probes in the Study of  
Anti-RT Activity**

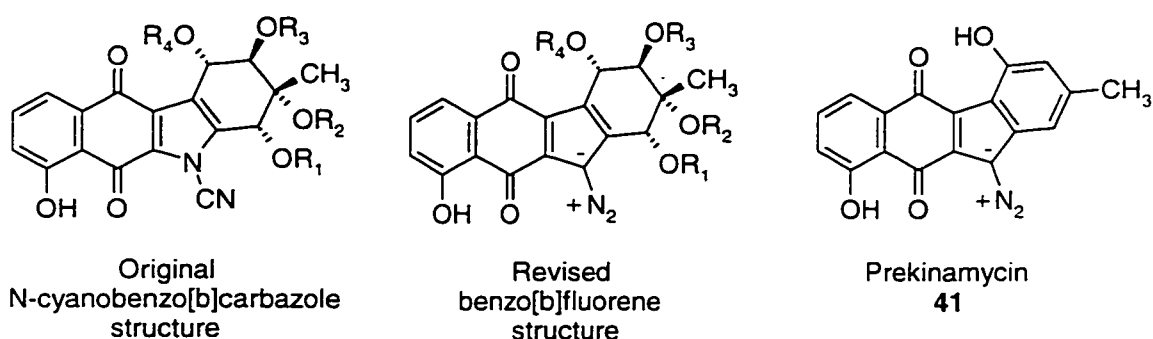


### 1.3 The Kinamycins and Related Benzo[b]fluorene Natural Products

It was also recognized in this group that the chemistry developed for the disubstituted naphthoquinones could be useful in another field of bioorganic chemistry. It was believed that it could be possible to synthesize mimics of kinamycins, a very interesting class of natural products, initially isolated from *Streptomyces murayamaensis* sp. nov. Hata and Ohtani, possessing antitumor antibiotics properties.<sup>3,4</sup> Kinamycins are one of the few benzo[b]fluorenes discovered in nature. The antibiotic property of kinamycin D, the most potent of all the kinamycins discovered so far, is very strong against Gram-positive bacteria but slightly so against Gram-negative bacteria.<sup>3,4</sup> These compounds also exhibit some antitumour activity. Since their discovery in 1970 and up to 1994, kinamycins and their precursors had been assigned an incorrect N-cyanobenzo[b]carbazole skeleton (Figure 15). This structure was proven to be incorrect by a former student in our research group, Salim Mithani, who synthesized unambiguously the N-cyanobenzo[b] carbazole that was believed to be prekinamycin, a biosynthetic precursor to the kinamycins.<sup>5</sup> After a thorough comparison of the spectroscopic properties found in literature for the prekinamycin **41** with the newly synthesized molecule, Mithani et al.<sup>5,41</sup> came to the conclusion that prekinamycin and kinamycins did not possess a N-cyanobenzo[b] carbazole ring system. This intriguing finding called for a review of all the chemical and spectroscopic analyses ever performed on this class of compound as well as on the published X-ray diffraction study of *p*-bromobenzoate of kinamycin C. In the original work, several unusual spectroscopic data concerning the cyano group could not be explained. Furthermore, the exact order of the linear three-atom fragment, two nitrogens and one carbon, at the location of the cyano group could not be assigned with certainty. Prekinamycin, as the kinamycins, were soon after

demonstrated by our research group to possess a diazo benzo[b]fluorene skeleton. The unusual spectroscopic data that could not be explained for the N-cyanobenzo[b] carbazole skeleton fit extremely well with a diazobenzo[b]fluorene skeleton. Gould and co-workers at Oregon State University independently came to the same structural conclusion by carrying out a very careful X-ray crystallographic analysis of the (S)-2-methylbutyric acid ester of kinamycin D.<sup>42</sup>

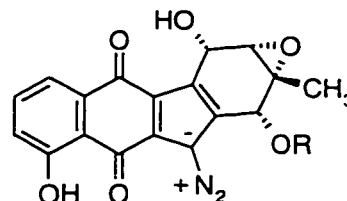
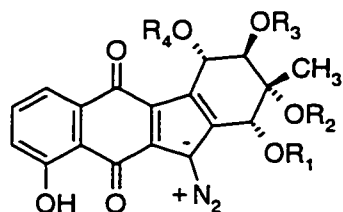
**Figure 15**



Up to now, 20 or so members of the benzo[b]fluorene class of natural products have been isolated. A good majority of them are either precursors of the kinamycins<sup>42</sup> **1 a-j** or are closely related to them. All of them seem to be polyketide-derived. They are prekinamycin<sup>43</sup> **41**, FL-120 A~D'<sup>44</sup> **42-47**, stealthin A-C<sup>45,46</sup> **48-50**, ketoanhydrokinamycin<sup>47</sup> **51**, kinobscurinone<sup>48</sup> **52**, cysfluoretin<sup>49</sup> **53**, momofulvenone A **54** and B **55**<sup>50</sup>, seongomycin<sup>51</sup> **56**, and kinafluorenone<sup>52</sup> **57**. Just like the kinamycins, most of these metabolites have displayed very interesting biological activities. Some of these compounds were discovered while trying to elucidate the biosynthetic pathway of the kinamycins.<sup>53</sup> As mutants of the original strain were generated, accumulations of several

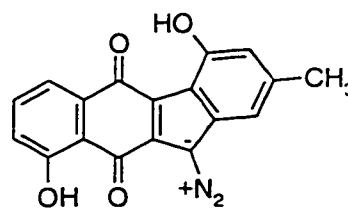
metabolites of kinamycins were obtained and characterized. The discovery of organisms producing new kinds of kinamycins has also been reported in the last few years.<sup>54</sup>

Figure 16

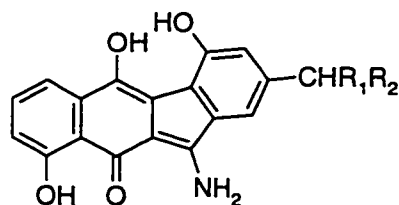


		<u>R<sub>1</sub></u>	<u>R<sub>2</sub></u>	<u>R<sub>3</sub></u>	<u>R<sub>4</sub></u>
1a	Kinamycin	A	H	Ac	Ac
b		B	H	Ac	H
c		C	Ac	H	Ac
d		D	Ac	H	Ac
e		E	Ac	H	H
f		F	H	H	H
g		G	Ac	COiPr	Ac
h		H	COiPr	H	Ac
i		I	COiPr	H	COiPr
j		J	Ac	Ac	Ac
42	FL-120 A		Ac	COiPr	Ac
45	FL-120 C		COiPr	H	Ac
46	FL-120 C'		COiEt	H	Ac
47	FL-120 D		COiPr	H	H

43 FL-120 B, R= Ac  
44 FL-120 B', R= COiPr

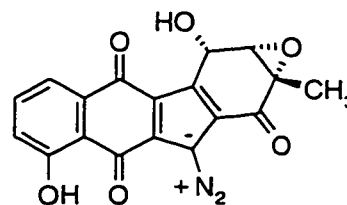


Prekinamycin  
41

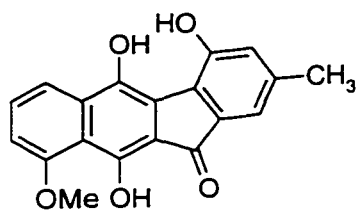


Stealthin

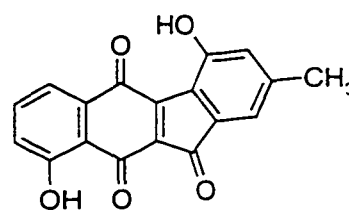
- 48 A R<sub>1</sub> = OH, R<sub>2</sub> = H  
49 B R<sub>1</sub> = R<sub>2</sub> = O  
50 C R<sub>1</sub> = R<sub>2</sub> = H



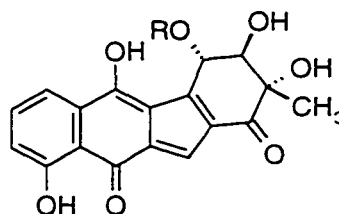
Ketoanhydrokinamycin  
51



Kinafluorenone  
57



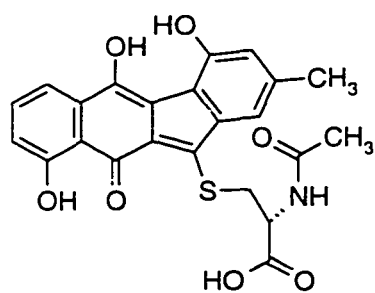
Kinobscurinone  
52



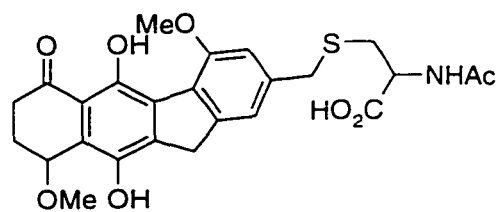
Momofulvenone

54 A R = H

55 B R = Ac



Seongomycin  
56



Cysfluoretin  
53

Stealthins A **48** and B **49** are potent radical scavengers produced by *Streptomyces viridochromogenes*.<sup>45</sup> In 1992, their structure was identified as the first ever reported natural benzo[b]fluorene skeleton. Research for novel free radical scavengers in the hope of ameliorating disease caused by oxygen-related free radicals, such as inflammation, atherosclerosis, Parkinson's disease, ischemic injuries to the central nervous system and cardiovascular system are underway. Just recently, stealthin C **50**, an aminobenzo[b]fluorene, has been identified as a new intermediate in the biosynthesis of kinamycin D **1d**.<sup>46</sup>

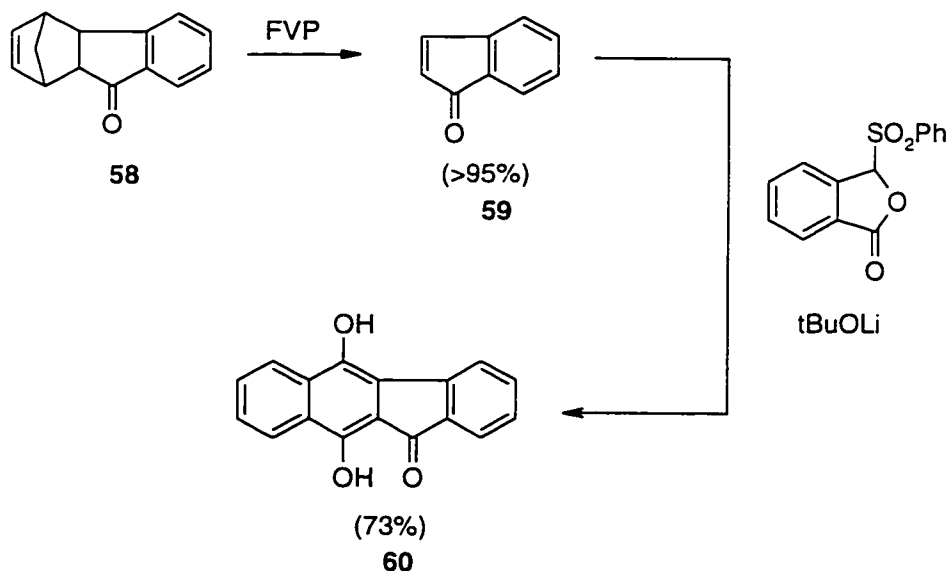
Seongomycin **56**, a sulfur-containing benzo[b]fluorene, and kinobscurinone **52**, a more advanced intermediate for the biosynthesis of kinamycin C and D, have been characterized by Gould and coworkers.<sup>51</sup> As well as these, 6 new kinamycins antibiotics, FL-120A-D **42-47**, produced by *Streptomyces chattanoogensis* subsp. *taitungensis* have been reported.<sup>44</sup> Their structures have been elucidated by 1D and 2D NMR spectroscopy and mass spectrometry.

Another example of a benzo[b]fluorene with a very interesting biological activity is cysfluoretin **53**. From a practical point of view, cysfluoretin is of interest as an inhibitor of glutathione S-transferase (EC 2.5.1.18) (GST)<sup>49</sup> since this enzyme plays an important role in the unwanted drug detoxication process of drug resistant tumor cells against anti-tumor agents such as anthracyclines, vinca alkaloids, and actinomycin D.<sup>54, 55</sup> No antimicrobial activity has been detected at 100 µg/mL and no toxic indications have been observed in mice after i.p. injection at a dose of 100 mg/kg.<sup>49</sup>

Momofulvenones A **54** and B **55** can be considered as the nitrogen-free parent compounds of the kinamycins. These two benzo[b]fluorene quinones were isolated from *Streptomyces diastatochromogenes* and had their structure determined in 1995.<sup>50</sup> For both of these compounds, no significant biological activity has been detected against bacteria or fungi.

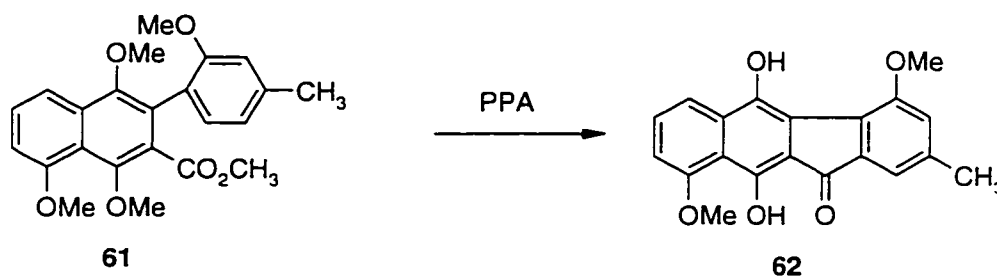
Several different synthetic approaches to the benzo[b]fluorene natural products, all involving a benzo[b]fluoren-11-one intermediate, have been explored. The annulation of indenone **58** with phthalide sulfone **59**, performed by Mal and Hazra,<sup>56</sup> afforded the benzo[b]fluorenone **60** with a yield of 73% (Scheme 21).

Scheme 21



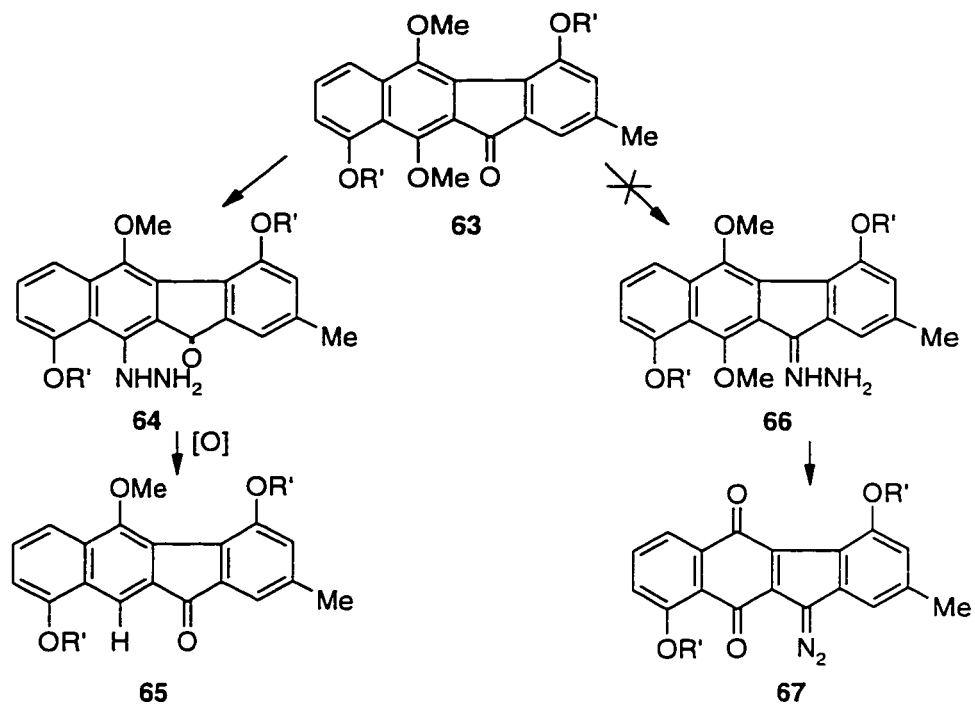
Gould and coworkers<sup>57</sup> were able to synthesize a benzo[b]fluoren-11-one intermediate by ring closure of the ester **61** with polyphosphoric acid to yield the unprotected quinone **62** (Scheme 22). A major drawback of using a benzo[b]fluoren-11-one intermediate is that the functionality at the C-11 seems to have an unusual reactivity. When Gould reacted the benzo[b]fluoren-11-one intermediate **63** with hydrazine, followed by oxidation in order to obtain the prekinamycin ring system **67**, the product obtained was **65** formed via the isolable hydrazine **64** (Scheme 23).

### Scheme 22

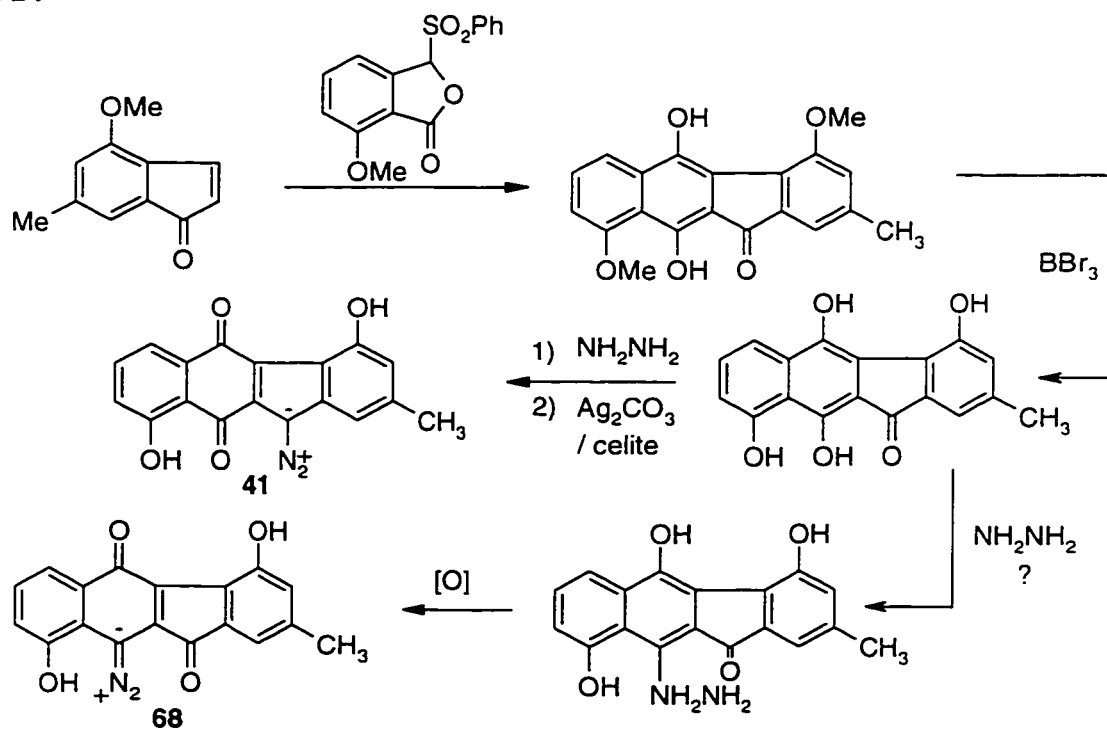


Hauser and coworkers<sup>58</sup> have combined the phthalide annelation methodology, similar to that reported by Mal and Hazra,<sup>56</sup> in order to synthesize prekinamycin regioselectively (Scheme 24). Since, as in the case of Gould,<sup>57</sup> hydrazine was used to convert the 11-one **63** to the diazo compound **41**, the Hauser synthesis of prekinamycin is not unambiguous since they did not disprove or consider the formation of the possible anomalous hydrazine observed by Gould (Scheme 23), which might lead in Hauser's synthesis to the 10-diazobenzo[b]fluorene **68** isomeric with prekinamycin.

Scheme 23

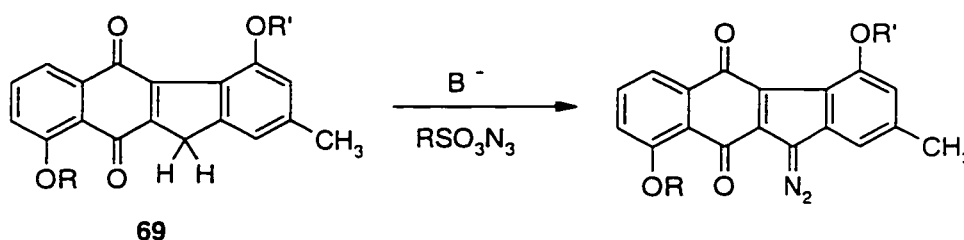


Scheme 24



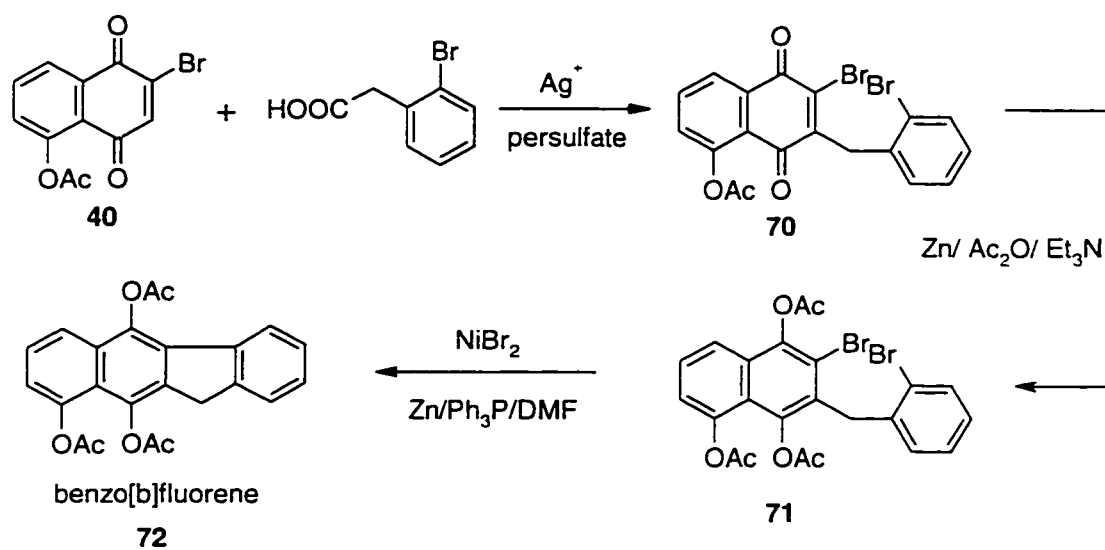
A possible solution to the regioselectivity issue in the introduction of the diazo group of prekinamycin, considered in this laboratory, involves the construction of a benzo[b]fluorene precursor **69**, in which the C-11 position is unsubstituted, followed by a base catalyzed diazotransfer reaction (Scheme 25).

**Scheme 25**



One approach to this problem has been examined in our laboratory (Scheme 26).<sup>5</sup> It has been shown that the 11H-benzo[b]fluorene skeleton **72** can be readily synthesized by a nickel-catalyzed cyclisation of 3-*o*-bromobenzyl-2-bromonaphthalene **71**. The present work represents model studies aimed at developing a variation of this approach to the 11H-benzo[b]fluorene system.

Scheme 26



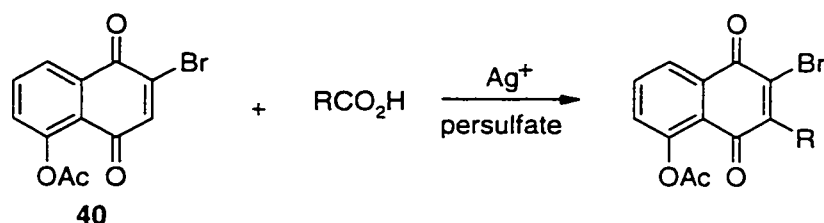
Some of the work described below was aimed at developing methods useful for the unambiguous synthesis of 11-diazobenzo[b]fluorenes.

## Chapter 2: Regioselective Alkylation of 1,4-Naphthoquinones

### 2.1 Previous Utilization of the Jacobsen-Thorssell Reaction

The interest in constructing 1,4-naphthoquinones for possible screening as inhibitors of HIV-1 RT and as possible intermediates in the synthesis of natural products related to the kinamycins, led to the exploration of methods for regioselective alkylation of the 1,4-naphthoquinone system. Previous studies in this laboratory by S. Mithani<sup>5</sup> demonstrated that the oxidative decarboxylation method for quinone alkylation developed by Jacobsen and Thorssell<sup>15</sup> as described in the introduction was applicable to alkylation of the bromoacetoxy-1,4-naphthoquinone, **40** (Scheme 27).

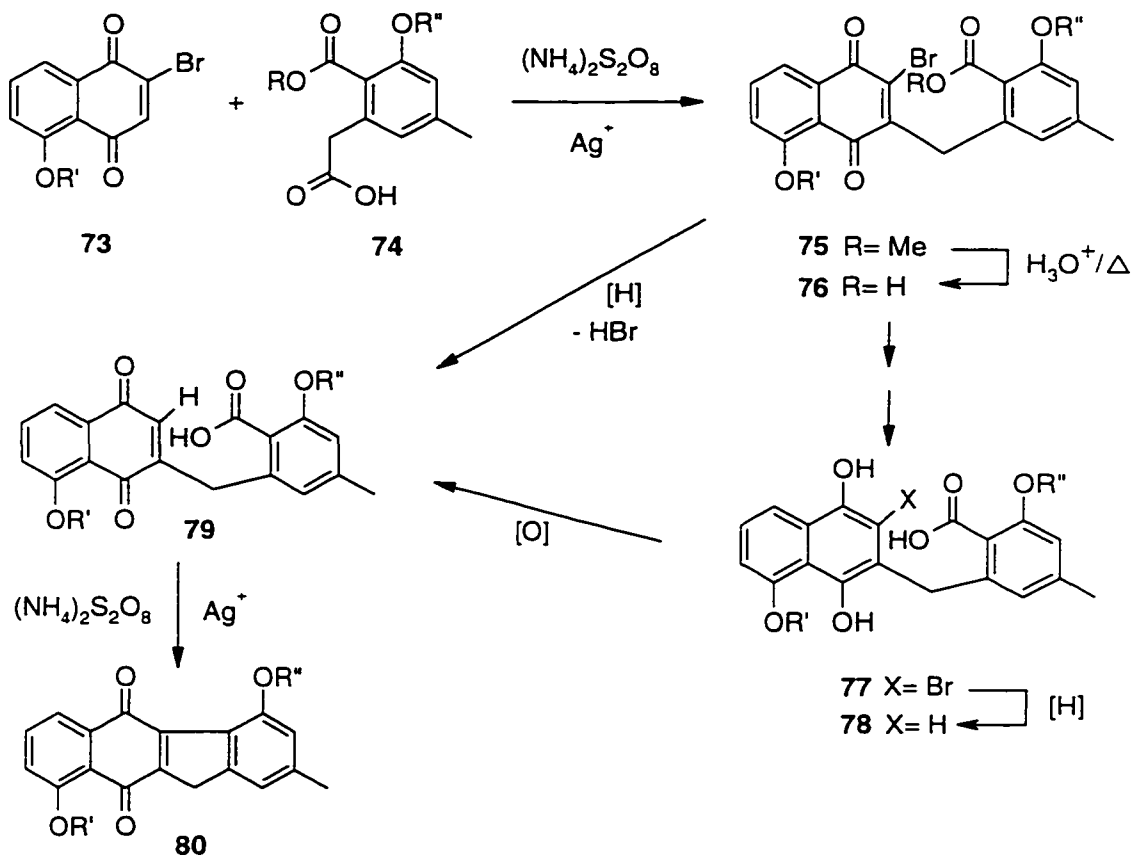
Scheme 27



As demonstrated in Scheme 26, alkylation by *o*-bromophenylacetic acid was found to give the quinone **70** in good yield and reduction of **70** with Zn in the presence of acetic anhydride and Et<sub>3</sub>N gave the triacetoxynaphthalene system **71**. It was also shown that **71** could be cyclised to the benzo[b]fluorene system **72** using Ni<sup>0</sup>, generated *in situ* by reduction of NiBr<sub>2</sub> by Zn. Although the cyclization was successful, it was found that the yield was low and that the reaction suffered from difficulties of irreproducibility. As a result, it was decided that an alternate route to the benzo[b]fluorene system found in the kinamycins should be

designed based on the assumption that both of the bonds to the naphthalene starting material might be constructed by use of the Jacobsen-Thorsell reaction (Scheme 28).

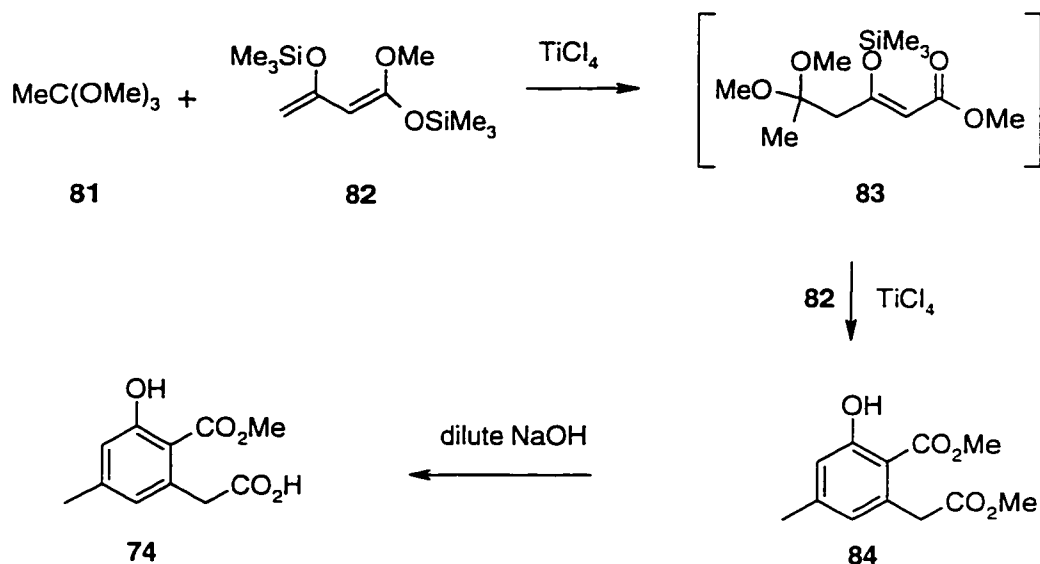
Scheme 28



It was felt that substituted phenylacetic acid **74** might be reacted with **73** under Jacobsen-Thorsell conditions to produce **75** regioselectively and that hydrolysis of the benzoate ester group in **75** would give **76**. Hydrogenolysis of the Br bond in **76** would then give **79**, either directly or by initial reduction to the hydroquinone **77** followed by hydrogenolysis to **78** depending on the choice of reducing agents. The fact that Chan and co-

workers<sup>59</sup> had reported a synthesis of the system **74** (Scheme 29) encouraged us in pursuit of this strategy.

Scheme 29

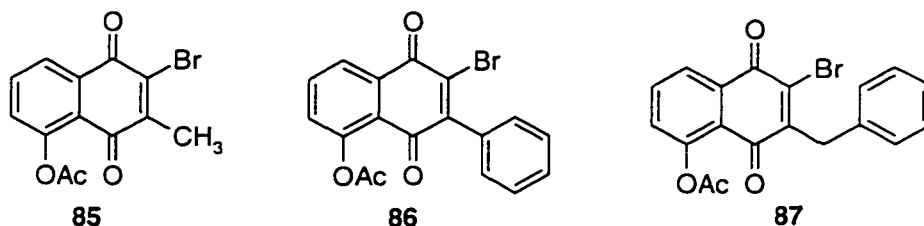


It was also felt that this stepwise regioselective bisalkylation sequence should be developed initially in an intermolecular context before attempting the intermolecular/intramolecular version shown above. The intermolecular alkylations also were expected to generate substituted naphthoquinones for screening as potential inhibitors for HIV-1 RT.

## 2.2 Monoalkylation/Arylation of 2-Bromo-5-acetoxy-1,4-naphthoquinone

In practice, it was found that difficulties were encountered in the initial mono-alkylation/arylation stage of 2-bromo-5-acetoxy-1,4-naphthoquinone when the reactions were performed on scales higher than those employed in the previous studies in this laboratory by S. Mithani.<sup>5</sup> Apart from an incomplete reaction, the alkylation products (Figure 17) were accompanied by highly colored polar byproducts which were assumed to be oligomeric.

**Figure 17**



The presence of unreacted quinone is a major complication in these reactions since, in order to proceed to the next step explained later, the product must be completely devoid of the starting material. Different purification methods such as column chromatography with different solvent systems and recrystallization were tried and proved to be unsuccessful. Considerable effort was then expended aimed at altering the reaction conditions to optimize the yields of these reactions and to push them to completion. Minor improvements were noted when a slower rate for the addition of a more dilute aqueous ammonium persulfate solution was used. An increase in the temperature, the reaction time, and the amount of carboxylic acid did not alter the outcome significantly and in some cases seemed to have a negative effect since hydrolysis of the 5' acetoxy group became more prevalent (Table 2).

**Table 2- Results of Various Jacobsen-Thorssell Reactions with Pure Acetonitrile as the Initial Solvent**

Acid used (equivalents)	quinone	AgNO <sub>3</sub>	(NH <sub>4</sub> ) <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	CH <sub>3</sub> CN/H <sub>2</sub> O present (mL./mL.)		Time / Temperature		Yields / comments	product
	(mmol)	(equ.)	(equ.)	before addition of persulfate	after addition of persulfate	for addition of persulfate	after the addition		
1. acetic acid (1.0)	1.78	0.27	2.6	15 / 0	15 / 18	5 min / 60°C	2 h / 95°C	Not complete (not pure)	<b>40 + 85</b>
2. acetic acid (1.0)	10.2	0.27	2.6	90 / 0	90 / 20	15 min / 60°C	4 h / 80°C	5% (pure after column) + ~ 21% (not complete)	<b>85</b> <b>40 + 85</b>
3. acetic acid (1.2)	1.69	0.33	3.2	12 / 0	12 / 14.5	1 h / 60°C	5 h / 95°C	16% (pure) + 63% of 5-OH 3-CH <sub>3</sub> (pure)	<b>40 + 85</b> <b>91</b>
4. acetic acid (1.2)	10.2	0.33	3.2	84 / 0	84 / 100	1.5 h / 60°C	5 h / 90°C	24% (pure) + 4% of 5-OH 3-CH <sub>3</sub> (pure)	<b>40 + 85</b> <b>91</b>
5. benzoic acid (1.2)	10.2	0.33	3.2	84 / 0	84 / 100	2 h / 60°C	5 h / 90°C	37% (pure)	<b>86</b>
6. phenylacetic acid (2.0)	6.85	0.50	4.0	60 / 0	60 / 72	2 h / 60°C	3 h / 95°C	18% (pure)	<b>87</b>

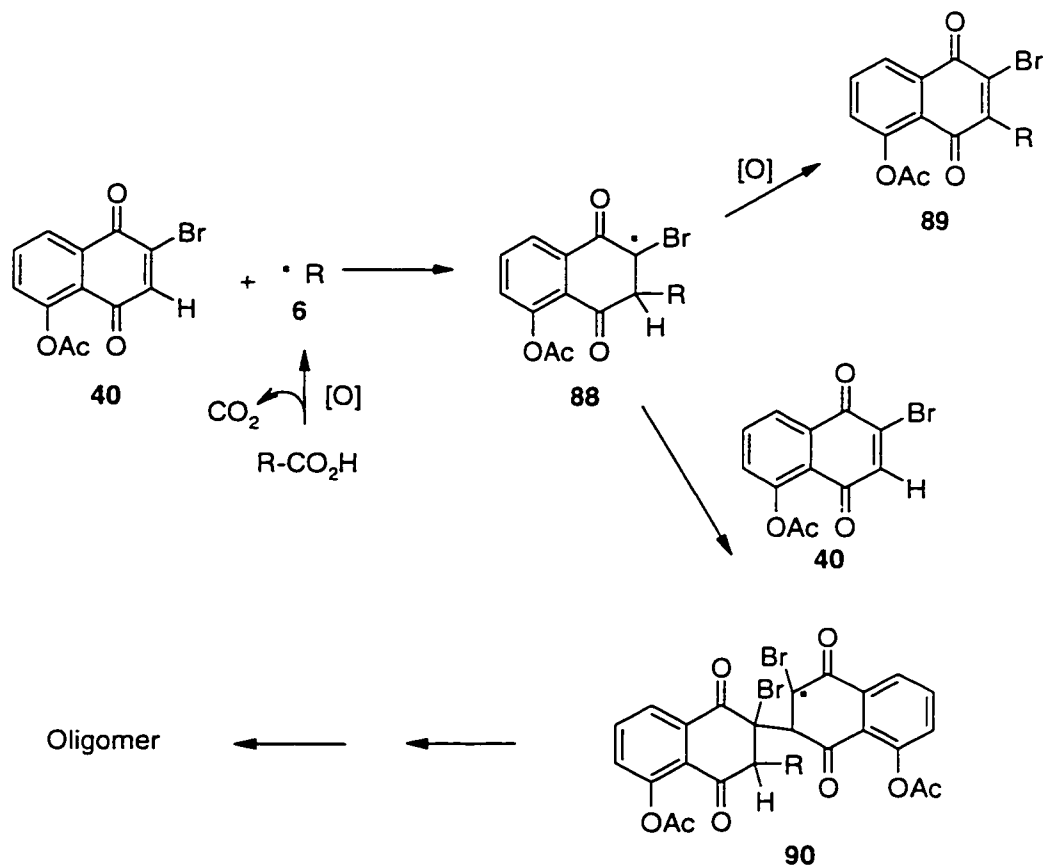
A critical examination of the reaction conditions employed in previous work in this laboratory was undertaken to find the source of the problem which resulted in apparent oligomerization of the starting quinone in competition with the desired alkylation. The conditions used previously employed a high ratio of organic co-solvent to aqueous component. In particular, the organic reactants were dissolved in acetonitrile and the aqueous persulfate oxidant was added dropwise to the hot solution.

A consideration of the possible unfavourable side reaction, the oligomerization as shown in Scheme 30, suggested that the use of such a high ratio of organic/aqueous medium in the initial stages of the reaction might be the cause of the difficulties. That is, the high proportion of the organic solvent component ensures full solubilization of the quinone making the quinone accessible for reaction with any radical such as **88** in an oligomerization sequence. On the other hand, the desired oxidation step **88**→**89** might be slowed down if the low water concentration results in poor solubilization of the inorganic oxidants. It was decided that conditions which decrease the solubility of the quinone and increase the solubility of the inorganic component might be useful.

It was found that when the temperature was lowered to 60°C in concert with the addition of water to the acetonitrile solution prior to the addition of the aqueous persulfate solution, the yields of the desired quinone product **89** improved dramatically and a reproducible route for the alkylation process on substantial scales was achieved. A ratio of  $\text{CH}_3\text{CN}/\text{H}_2\text{O} = 1:3$  was found to give optimal results. Addition of more silver nitrate midway in the addition of the persulfate had a positive effect by pushing the reaction in favour of the

desired product. From this last observation, the suggestion that inactivation of the silver catalyst either by light or by some poison is raised.

Scheme 30



By combining all the findings just mentioned, an improved version of the Jacobsen-Thorsell method aimed specifically toward the alkylation of naphthoquinones was obtained. A number of products were generated in this way, as shown in Table 3.

**Table 3- Results of Various Jacobsen-Thorsell Reactions with a Mixture of Acetonitrile and Water as the Initial Solvent**

Acid used *	quinone (mmol)	AgNO <sub>3</sub> (equ.)	(NH <sub>4</sub> ) <sub>2</sub> S <sub>2</sub> O <sub>8</sub> (equ.)	CH <sub>3</sub> CN/H <sub>2</sub> O present (mL / mL)		Time / Temperature		Yields	product
				before addition of persulfate	after addition of persulfate	for addition of persulfate	after the addition		
1. acetic acid	1.69	0.3	1.1	2.1 / 6.3	2.1 / 8.0	4 h / 60°C	10 min / 60°C	45% complete	<b>40 + 85</b>
2. The product isolated from reaction 1 was subjected to the same conditions using 1.5 mmol of AcOH								70% complete	<b>40 + 85</b>
3. The product isolated from reaction 2 was subjected to the same conditions using 1.2 mmol of AcOH								80% complete	<b>40 + 85</b>
4. acetic acid	5.08	1.0	3.0	6.3 / 19.0	6.3 / 24.1	45 min / 60°C	10 min / 60°C	87% final yield	<b>85</b>
5. benzoic acid	1.69	0.3	1.1	2.1 / 6.3	2.1 / 8.0	2 h / 60°C	10 min / 60°C	25% complete	<b>40 + 86</b>
6. benzoic acid	1.69	0.3	3.0	15.0 / 45.0	15.0 / 49.7	2 h / 60°C	20 min / 60°C	90% complete	<b>40 + 86</b>
7. The product isolated in reaction 6 was subjected to the same conditions but using 0.60 mmol of PhCO <sub>2</sub> H								72% final yield	<b>86</b>
8. phenylacetic acid	1.69	0.3	1.1	2.1 / 6.3	2.1 / 8.0	30 min / 60°C	10 min / 60°C	80% complete	<b>40 + 87</b>
9. phenylacetic acid	1.69	0.3	1.1	2.1 / 6.3	2.1 / 8.0	3 h / 60°C	1 h / 60°C	85% complete	<b>40 + 87</b>
10. The product from reaction 9 was subjected to the same conditions but using 0.74 mmol of phenylacetic acid								96% final yield	<b>87</b>

\* 1.7 equivalents of acid were used unless otherwise specified

For the reaction of **40** with acetic acid using an aqueous acetonitrile system at the outset of the reaction (entry #1, Table 3) using 0.3 equivalent of  $\text{AgNO}_3$ , a clean reaction without the formation of highly coloured oligomers was observed producing a 55:45 mixture of **40** and the alkylation product **85**. Recycling this mixture (entry #2 and 3, Table 3) under the same condition increased the % conversion to 80%. When a full equivalent of  $\text{AgNO}_3$  and a 3 equivalent of persulfate were employed (entry #4, Table 3) the reaction proceeded cleanly to give an 87% isolated yield of **85** in a single step.

For benzoic acid, the reaction was only 25% complete using a 1:2 ratio of  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  at the outset of the reaction with 0.3 equivalent of  $\text{AgNO}_3$  and 1.1 equivalents of persulfate (entry #5, Table 3). Increasing the total amount of the solvent approximately seven fold without altering the acetonitrile/water ratio and increasing the persulfate amount to 3.0 equivalents led to a reaction which was 90% complete (entry #6, Table 3). One recycling of this product resulted in complete consumption of the starting quinone and a 72% isolated yield of the C-3 phenyl naphthoquinone **86** (entry #7, Table 3).

With phenylacetic acid using a 1:3  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  initial solvent system, 0.3 equivalents of  $\text{AgNO}_3$  and 1.1 equivalents of persulfate the reaction was 80% complete after 40 minutes (entry #8, Table 3). Under the same conditions, the reaction was observed to be 85% complete after 4 hours (entry #9, Table 3). Recycling this product (entry #10, Table 3) produced **87** in 96% isolated yield.

It should be pointed out that for each of the acids studied, conditions have been found which result in a clean conversion to a product containing 80-90% of the desired alkylation product accompanied by 20-10% of the starting quinone. If the chromatographic separation of the alkylated quinones and the starting quinone had been effective, the recycling steps described above which make this method somewhat cumbersome would not have been necessary. Thus, for other alkylations in which the product and starting materials are more readily separated a one step alkylation should be more readily achievable.

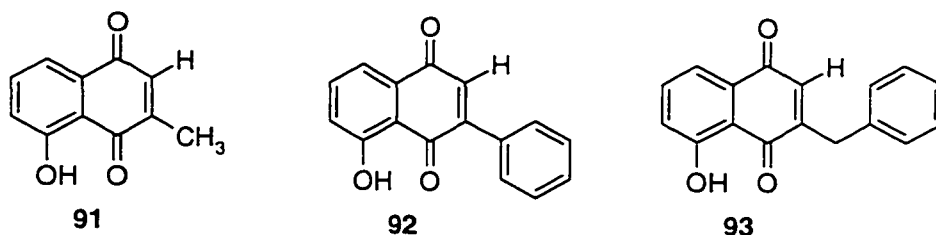
In any event, the availability of pure samples of **85**, **86**, and **87** allowed for exploration of the reductive debromination-alkylation sequence analogous to that shown in Scheme 28, which is described above.

### 2.3 Reductive Debromination

With the initial monoalkylation procedure worked out, attention was turned to the reductive debromination process. The purpose of this second step is to replace the bromine at the 2 position with the hydrogen needed to perform a second alkylation, at that position, using the Jacobsen-Thorssell method. As stated briefly earlier, no bromo quinone unsubstituted at the 3 position must be present at this point since the debromination of the starting material would afford a quinone with two sites for the second alkylation.

Different methods to debrominate the bromoquinone directly were tried. Treatment with an acidic aqueous solution of  $\text{SnCl}_2$ <sup>60</sup> followed by oxidation with  $\text{CrO}_3$  resulted in poor yields of complex mixtures (several spots on the TLC). From  $^1\text{H-NMR}$  analysis it seems that the dehalogenation worked for all 3 substituted quinones (3-methyl, 3-phenyl, and 3-benzyl) since the expected peak at around 7 ppm, corresponding to the new proton at the 2 position, was obtained. No peaks were seen for the acetoxy group (around 2.45), however. Instead, a singlet at  $\delta > 10$  ppm was observed in each case suggesting that the product might contain the deacylation product, i.e. the phenols **91-93** corresponding to the desired product. This method was not pursued further.

**Figure 18**



Another method which has been described in the literature for reductive dehalogenation uses tri-*n*-butyltin hydride and a radical initiator.<sup>61</sup> For the present case the reaction might be expected to proceed as shown below (Scheme 31).



Scheme 32

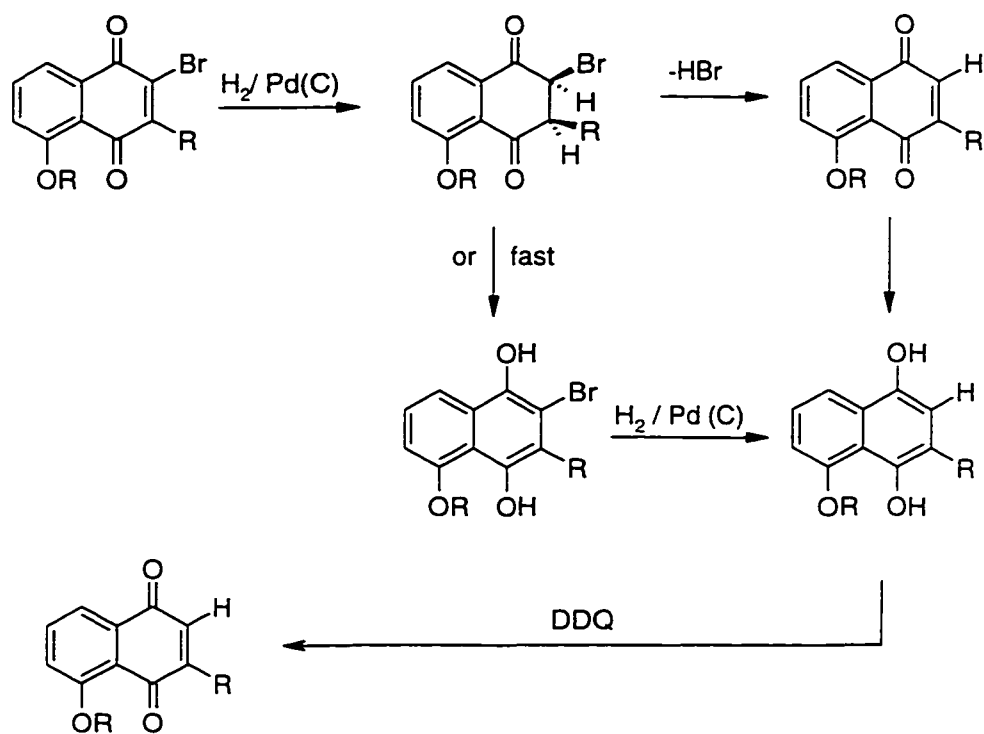
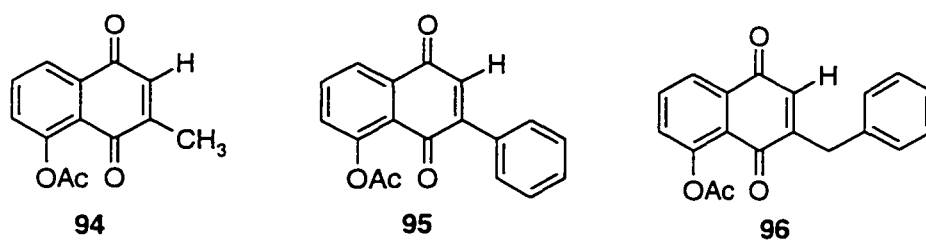


Figure 19



**Table 4- Debromination with H<sub>2</sub>Pd/C on 2-Bromo 3-substituted Quinones**

substitution at the 3 position / molecule	quinone (mmol)	solvent(s)	10% Pd/C (mg)	time	yields	product
1. Methyl / <b>85</b>	3.88	150 mL AcOH	400	1 day	96%	<b>94</b>
2. Methyl / <b>85</b>	0.38	50 mL AcOH	20	1 day	95%	<b>94</b>
3. Phenyl / <b>86</b>	0.27	150 mL AcOH	50	4 days	84%	<b>95</b>
4. Phenyl / <b>86</b>	0.62	150 mL AcOH	100	2 days	~ 65% to completion	<b>86 + 95</b>
5. The product isolated in reaction 4 was subjected to the same conditions				4 days	80%	<b>95</b>
6. Benzyl / <b>87</b>	3.17	130 mL AcOH	300	3 days	95%	<b>96</b>

All of these experiments were performed at a pressure of 50 psi.

2 equivalents of NaOAc were present during the dehalogenation.

As part of the workup, the debrominated hydroquinone obtained was oxidized to the corresponding quinone with 2 equivalents of DDQ.

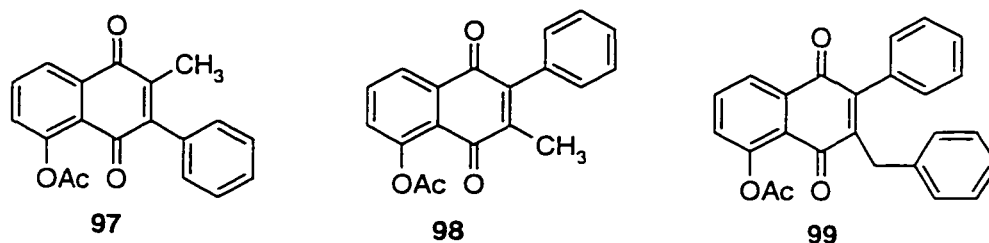
In general, acetic acid was found to be an appropriate solvent with 2 equivalents of NaOAc added to scavenge the HBr generated in the reaction and an H<sub>2</sub> pressure of 50 psi gave a reasonable reaction rate. In the case of the 3-phenyl-substituted quinone system the reaction proved to be sluggish and required 4 days to go to completion. It was found that this system exhibited poor solubility in acetic acid but addition of ethyl acetate to solubilize the starting material did not improve the rate of reaction. It would seem that the 3-phenyl system is intrinsically less reactive than the other quinones studied likely as a result of the resonance stabilization experienced in the conjugated aryl substituted quinone. In all cases, the oxidation of the initially formed hydroquinone by DDQ proceeded smoothly and the product was purified readily by chromatography on silica gel.

#### **2.4 Alkylation/Arylation of Debrominated Naphthoquinones**

Once the dehalogenation had been performed, the presence of a hydrogen now at the 2 position enabled us to perform the second coupling in a similar manner as for the 3 position using the Jacobsen-Thorsell method (Figure 20) (Table 5). Yields for this coupling were found, however, to be lower than for the first coupling. This might be caused by the fact that the hydrogen at the 2 position is more sterically hindered than was the one at the 3 position during the first coupling. However, the best yield was obtained from the reaction of the 3-benzyl-1,4-naphthoquinone with benzoic acid which should have represented the sterically most crowded transition state. Another possibility is that the solubility of the quinone has changed due to the group at the 3 position and the loss of the bromine. Since solubility of the

starting material and oxidant appears to have affected the yields in the Jacobsen-Thorsell reactions with the bromoquinone **40** it is possible that optimization of the acetonitrile/water ratio may be necessary for each of the compounds studied.

**Figure 20**



In summary, it has been shown that the stepwise alkylation/arylation-dehalogenation-alkylation/arylation sequence can yield unsymmetrically 2,3-disubstituted 5-acetoxy-1,4-naphthoquinone in a regioselective fashion.

**Table 5- Results of Jacobsen-Thorsell Reactions Performed on the Debrominated Substituted Quinones**

Acid used *	quinone  (mmol)	AgNO <sub>3</sub>  (equ.)	(NH <sub>4</sub> ) <sub>2</sub> S <sub>2</sub> O <sub>8</sub>  (equ.)	CH <sub>3</sub> CN/H <sub>2</sub> O present (mL / mL)		Time / Temperature		Yields	product
				before addition of persulfate	after addition of persulfate	for addition of persulfate	after the addition		
1. acetic acid	0.49 mmol of <b>95</b>	0.3	3.0	10 / 30	10 / 31.5	2 h / 60°C	30 min / 90°C	~ 95% complete	<b>92 + 97</b>
2. The product isolated in reaction 1 was employed again with 0.049 mmol of acetic acid		1.0	3.0	20 / 20	20 / 21.5	4 h / 60°C	30 min / 90°C	46% final yield	<b>97</b>
3. benzoic acid	1.59 mmol of <b>94</b>	0.5	3.0	4.7 / 14	4.7 / 18.3	30 min / 60°C	10 min / 90°C	~ 45% complete	<b>91 + 98</b>
4. The product isolated in reaction 3 was employed again with 0.80 mmol of benzoic acid		1.0 **	3.0	same	same	2.5 h / 60°C	10 min / 90°C	33% final yield	<b>98</b>
5. benzoic acid	0.59 mmol of <b>96</b>	1.0	3.0	7 / 5	7 / 5.6	1 h / 60°C	20 min / 60°C	~ 33% complete	<b>96 + 99</b>
6. The product isolated in reaction 5 was employed again with 0.44 mmol of benzoic acid		same	same	10 / 5	10 / 6.5	2 h / 60°C	20 min / 90°C	93% final yield	<b>99</b>

\* 2 equivalents of acid were used unless otherwise specified

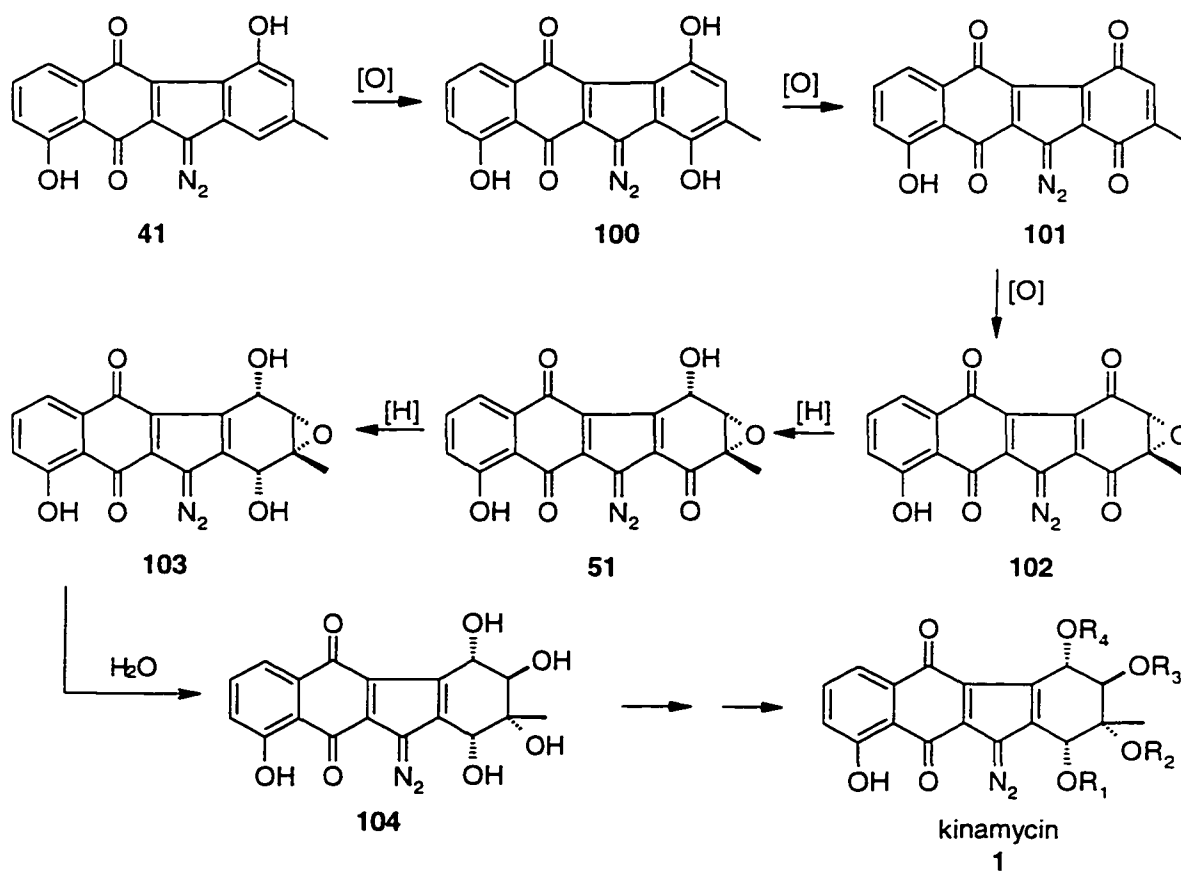
\*\* Half of the AgNO<sub>3</sub> was added before the persulfate and another half in the middle of the addition of the persulfate

## Chapter 3: Model Studies Towards the Synthesis of the Kinamycin Type Antitumour Antibiotics

### 3.1 Model Studies Related to the Construction of the D-ring of the Kinamycins

The isolation of prekinamycin<sup>43</sup> **41**, ketoanhydrokinamycin<sup>47</sup> **51**, and O-acyl derivatives of the epoxy diol<sup>44</sup> **103** from kinamycin producing strains of *Streptomyces* has led to the suggestion of the following biogenetic sequence for the construction of the D-ring of the kinamycins (Scheme 33).

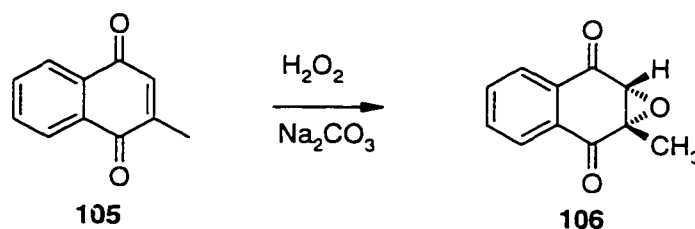
Scheme 33



In this sequence, monooxygenation of **41** gives the hydroquinone **100** which upon dehydrogenation to the quinone and epoxidation gives **102**. Stepwise reduction of the keto groups gives **51** and then **103** and nucleophilic ring opening of the epoxide **103** gives **104**. As a result, we became interested in exploring reactions which might be employed in synthesis of the kinamycin D ring in a more-or-less biomimetic fashion. In particular, we were interested in finding conditions which would effect the stereoselective ketone reductions. It was felt that once that stereoselectivity was established, the stereoselective epoxide ring-opening would be straight forward.

To explore this reduction, a simple model epoxide **106** was prepared by a method described many years ago by Fieser<sup>63</sup>.

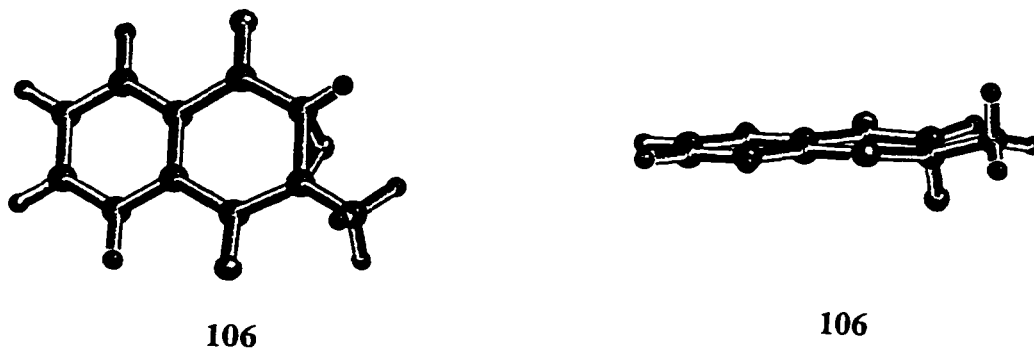
#### Scheme 34



Construction of a model of **106** (see Figure 21 below) suggested that the face of the keto groups opposite to the epoxide group was more accessible to the hydride reducing agents. It was decided that sodium borohydride<sup>64</sup> would be an appropriate reducing agent since the chances of reductive ring opening of the epoxide would be diminished relative to

those for LAH reduction and, since  $\text{NaBH}_4$  is known to reduce ketones in the presence of diazo groups<sup>65</sup> which would be a concern in the biomimetic synthesis.

Figure 21



In practice, reaction of **106** with 1 mole equivalent of  $\text{NaBH}_4$  resulted in reduction of both keto groups without reductive ring opening of the epoxide as desired. TLC,  $^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR analysis revealed that a single product was produced. The benzylic hydrogens in **107** give rise to two multiplets at  $\delta$  7.49-7.41 ppm (2 H) and at 7.27-7.20 ppm (2). The epoxide C-H appears as a singlet at 3.33 ppm where the two other cyclic hydrogens give rise to two doublets at 4.80 ( $J=6.9$ , 1 H) and at 4.65 ( $J=8.2$ , 1H). The two hydroxyl hydrogens appear as a multiplet at 5.80-5.73 (2H) and the  $\text{CH}_3$  group as a singlet at 1.47 ppm.

The stereochemistry of the epoxy diacetyl derivative **108** which is described below was established by difference nOe studies at 500 MHz.

Scheme 35

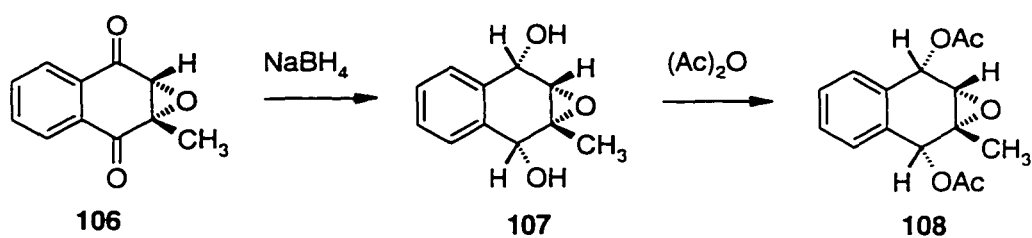
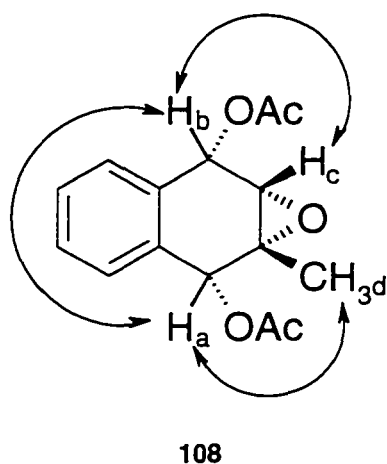


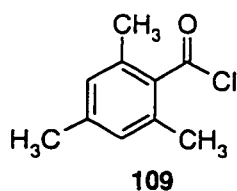
Figure 22



As shown in the Figure 22 above, irradiation of  $H_b$  gave positive nOe's for both  $H_a$  and  $H_c$  suggesting that all three non-aromatic ring hydrogens are present on the same face of the ring. As expected, a positive nOe was obtained for  $H_b$  when  $H_a$  was irradiated. Positive nOe's are also observed for both  $H_a$  and  $H_c$  upon the irradiation of the methyl hydrogens  $H_d$  supporting the idea that the methyl group is on the same side of the ring as  $H_c$  and therefore the same side of the ring as  $H_a$  and  $H_b$ .

Previous to the diacetylation of the diol, generated stereoselectively as described above, attempts were made to explore further transformations which might be useful in the eventual synthesis of the kinamycin D ring. It was hoped that the environments of the hydroxyl groups in **107** might be sufficiently different that selective acylation might be effected so that manipulation of the hydroxyl groups, including oxidation, could be carried out selectively. In practice, reaction of **107** in methylene chloride at room temperature with 1 equivalent of acetic anhydride and 2 equivalents of pyridine for 1 day gave a mixture of compounds which appeared to contain the starting material, the diacetyl derivatives and two mono acetyl derivatives. With longer reaction times (3 days), excess acetic anhydride (5 equivalents) and pyridine (8 equivalents), it proved possible to convert **107** into the diacetate **108**. From this study, it is clear that the accessibility of the two hydroxyl groups to acylating agents is comparable, at least with small acyl groups such as acetyl. It is suggested that more bulky acylating agents eg. mesitoyl chloride **109** be employed in effecting selective acylation of this system.

**Figure 23**



In addition, very preliminary attempts were made to effect stereoselective nucleophilic ring opening with oxygen nucleophiles such as acetate, employing Aliquat 336 in acetonitrile,<sup>66</sup> and with sodium methoxide.<sup>67</sup> The system was found to be inert to acetate

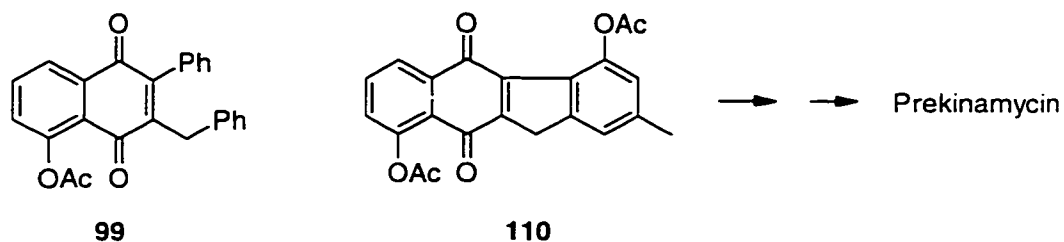
and to water in refluxing dioxane and to be unstable in the presence of methoxide in methanol, yielding a complex mixture of products which were not characterized. Future work in this area might employ more polar solvents (e.g. HMPA, DMSO) to increase the nucleophilicity of the oxygen nucleophile.

In summary, it has been shown that the epoxydiketone system **106** undergoes a stereoselective reduction to the diol **107** which possesses the relative stereochemistry found in the D ring of desacetyl FL-120B **103** which is proposed as a biosynthetic intermediate to the kinamycins. This chemistry may prove useful in the eventual biomimetic synthesis of kinamycins.

### 3.2 Model Studies of the Diazo Transfer Reaction

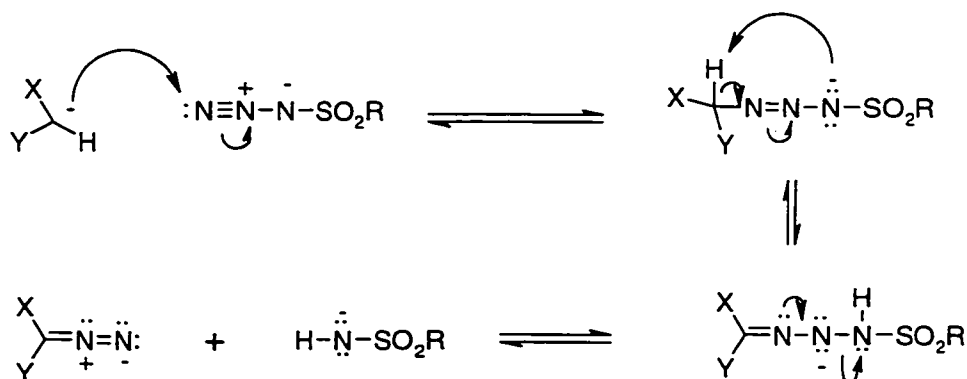
In connection with our interest in the kinamycins, we were concerned, not only with methods for constructing the carbon skeleton of the natural product, but also with the introduction of the diazo-functionality which gives the kinamycins their particular structural novelty and which likely plays a role in their antibiotic activity. Thus, with the method for alkylation of the naphthoquinones via the Jacobsen-Thorssell<sup>15</sup> method worked out as described above, attention was turned to the problem of introduction of the diazo group. The model system **99** was selected as a model for **110** which might serve as an intermediate in the construction of prekinamycin.

## Scheme 36



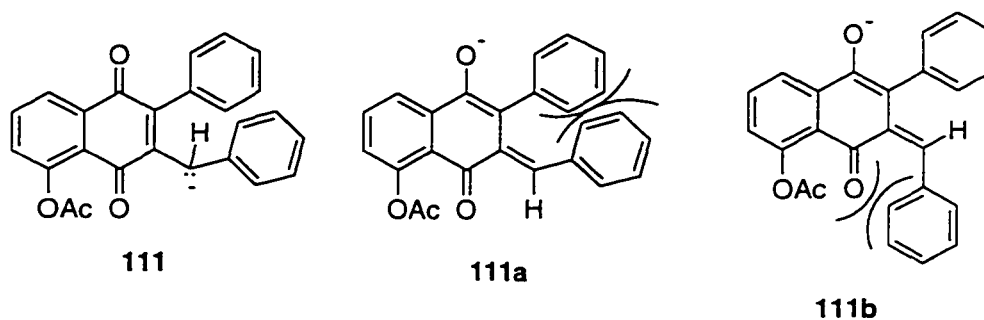
Since the  $\text{CH}_2\text{-Ph}$  group is attached to the quinone ring, these hydrogens were expected to be moderately acidic. Examination of the literature concerning the synthesis of diazo compounds reveals that activated methylene compounds  $\text{X-CH}_2\text{-Y}$  are readily converted into diazo compounds  $\text{X-CN}_2\text{-Y}$  by reaction with sulfonyl azides (e.g. tosyl azide) under basic conditions. This so-called diazo transfer reaction involves a deprotonation followed by nucleophilic attack on the azido group followed by proton shift and expulsion of the deprotonated sulfonamide.<sup>65</sup>

## Scheme 37



In practice, it was found that reaction of **99** under such condition with tosyl azide resulted only in recovery of starting material. As a result, it was felt that the acidity of the benzylic hydrogen was adversely affected (decreased) by the presence of the bulky C-2 substituent.

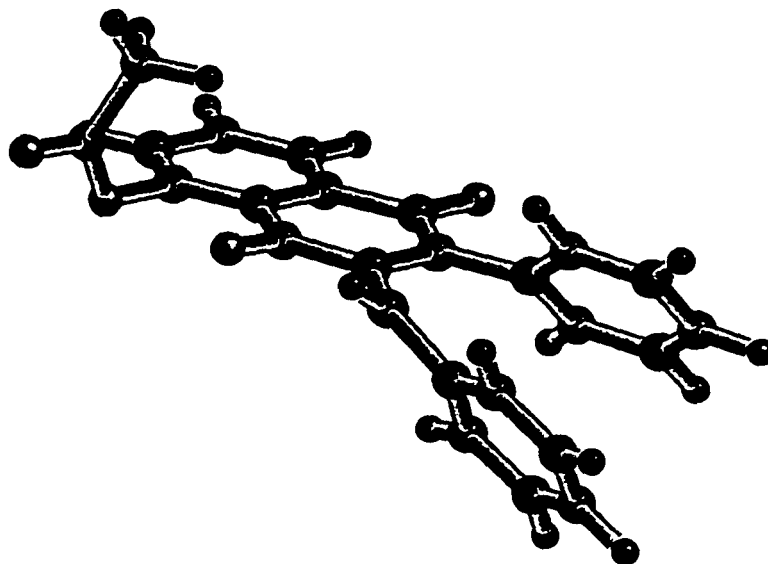
**Figure 24**



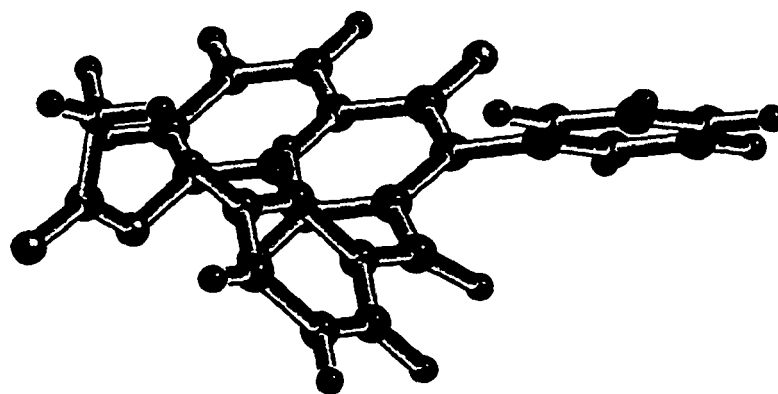
Construction of a molecular model of the enolate **111** using a molecular mechanics energy minimization suggests that the presence of the aryl group at C<sub>2</sub> prevents the enolate **111** from achieving full planarity (Figure 25). Similarly, the carbonyl group oxygen at C<sub>4</sub> prevents the planarity in the other possible stereoisomer of the enolate.

Since such a steric effect would play no part in the chemistry of the intermediate **110** in prekinamycin synthesis, it was decided that **99** was a poor model system and that the system **93** lacking the C-2 group should be examined (Scheme 38).

Figure 25

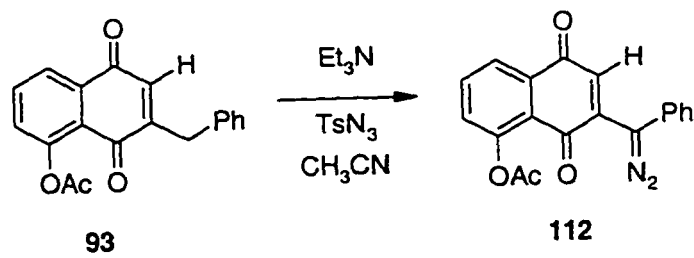


111a



111b

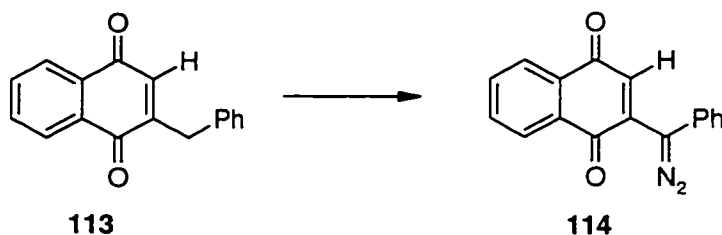
Scheme 38



In this case the desired diazo compound **112** was produced in 40% yield. That the diazo group was present in **112** was indicated by the characteristic I.R. band at  $\nu = 2097 \text{ cm}^{-1}$  and a  $^{13}\text{C}$  NMR signal at  $\delta 66.7 \text{ ppm}$  corresponding to the diazo group carbon atom. In prekinamycin diacetate the diazo IR band is at  $\nu = 2119 \text{ cm}^{-1}$  and the  $^{13}\text{C}$  NMR signal at  $\delta = 83.7 \text{ ppm}$ .<sup>5</sup>

It is worth noting that during the course of this project, a report appeared from Jebaratnam and co-workers<sup>68</sup> in which the related diazo compound **114** was also successfully converted into a diazo product as shown below. Of particular interest to us was our observation that the diazotransfer conditions were compatible with the acetoxy group in **93** since such a group would be present in the synthetic route planned for the natural product.

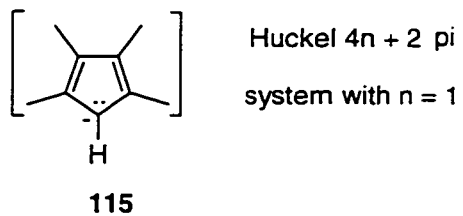
### Scheme 39



The fact that **112** in our work and **113** in Jebaratnam's work undergo a facile diazotransfer reaction whereas **111** does not is not a major concern for the proposed total synthesis of prekinamycin, since the steric problem present in **111** will be unimportant in the benzo[b]fluorene system which is flat. However, it is also expected that the anion **115** will be stabilized by aromatic character (cyclopentadienide anion-like) and, hence, might be less

reactive than the anion from **93** and **113**. This may require somewhat more vigorous reaction conditions for successful diazotransfer, although, the fact that diazo compounds can decompose thermally must also be borne in mind in future work.

**Figure 26**

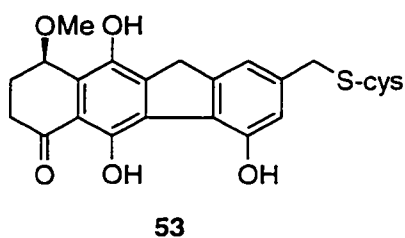


## Chapter 4: Model Studies Towards the Synthesis of Cysfluoretin

### 4.1 Building of the Ring System of Cysfluoretin via a Diels-Alder Addition

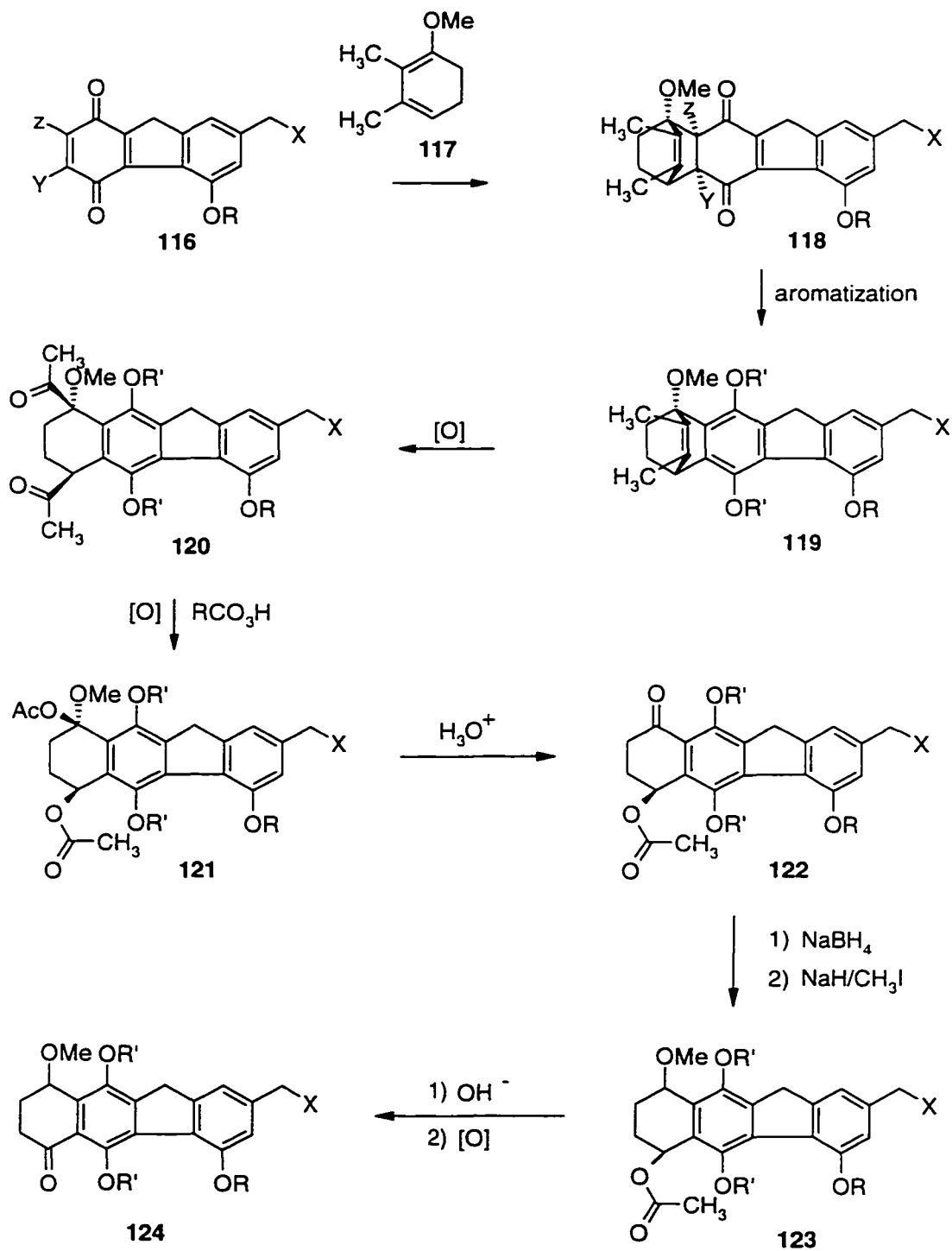
As mentioned in chapter 1, among the recently discovered natural products possessing a benzo[b]fluorene hydroquinone skeleton is cysfluoretin (**53**), isolated by Aoyama et al.<sup>49</sup> from *Streptomyces* sp. MI384-DF12. From a practical point of view, cysfluoretin is of interest as an inhibitor of glutathione S-transferase in tumor cell treatment.<sup>54, 55</sup>

Figure 27



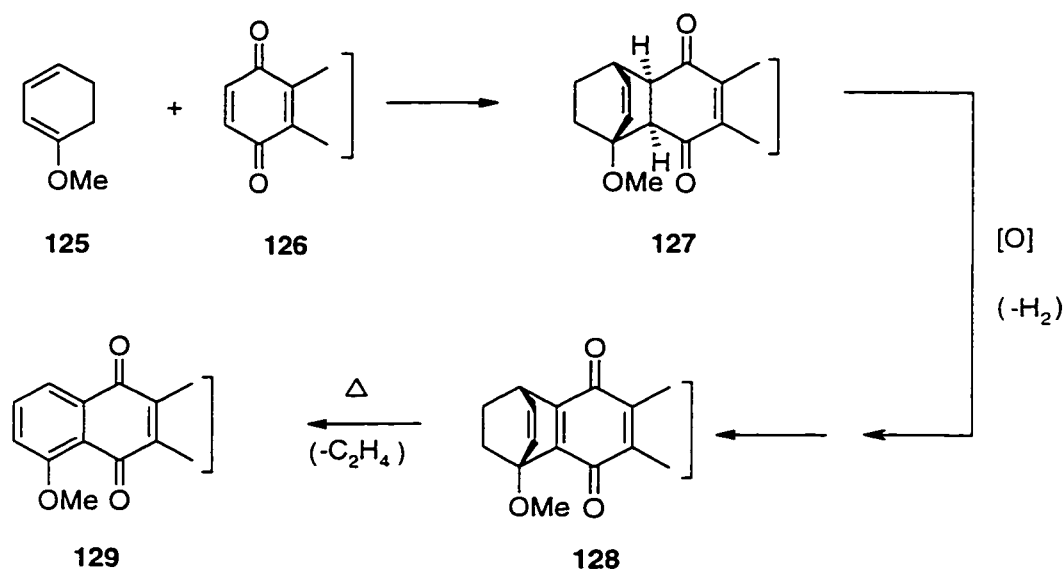
From a purely academic point of view, this ring system represents an interesting synthetic challenge. In this laboratory it was decided that the unusual A-ring system of this natural product might be constructed from a benzoquinone precursor such as **116** which possesses the B, C, and D rings of the natural product (Scheme 40).

## Scheme 40



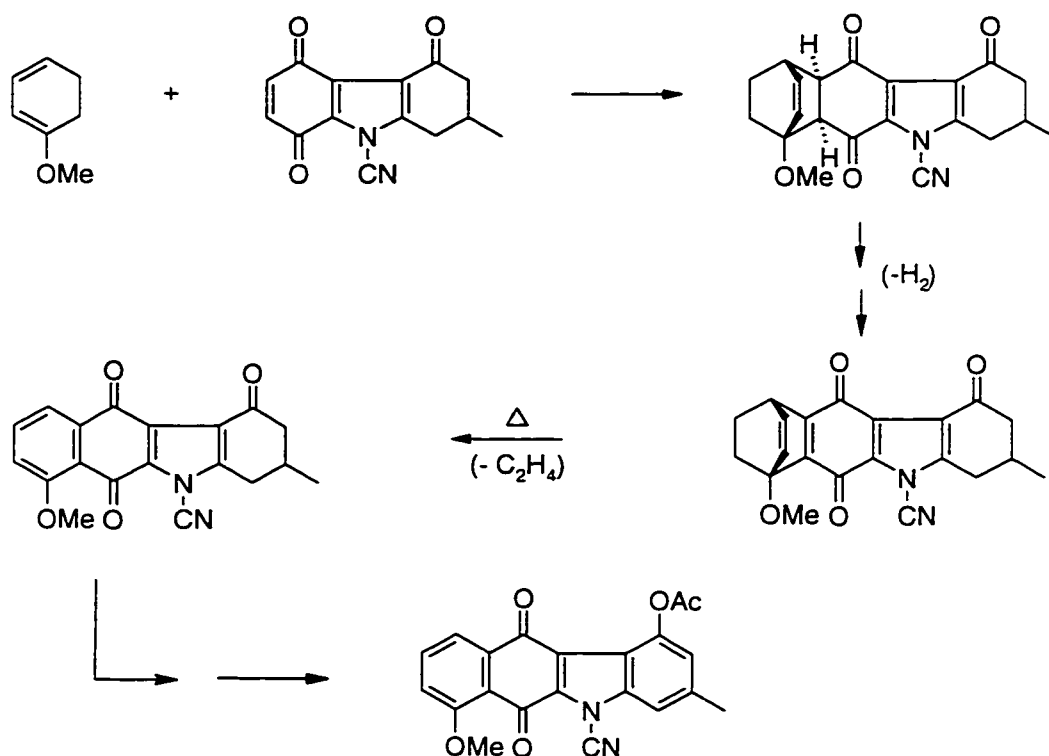
The success of the reaction sequence above depends on the regioselectivity of the cycloaddition process in the first step. The synthetic elaboration of *p*-benzoquinones **129** by means of Diels-Alder reaction with 1-methoxy-1,3-cyclohexadiene **125** was first reported by Birch and co-workers (Scheme 41).<sup>69</sup>

**Scheme 41**



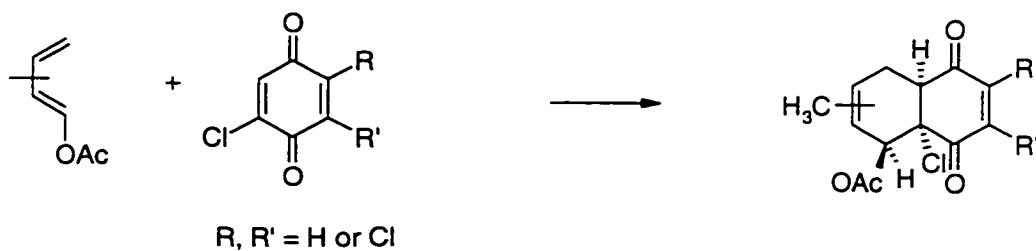
In their work, the Diels-Alder adduct **127** was used as a precursor to a methoxy benzene ring fused to the *p*-benzoquinone system. This was aromatized by oxidation of the diketone **127** to the benzoquinone **128** followed by thermal retro-Diels-Alder elimination of ethylene. In our own laboratory, a similar procedure was used to build the A-ring of the structure originally assigned to prekinamycin (Scheme 42).<sup>41</sup>

Scheme 42



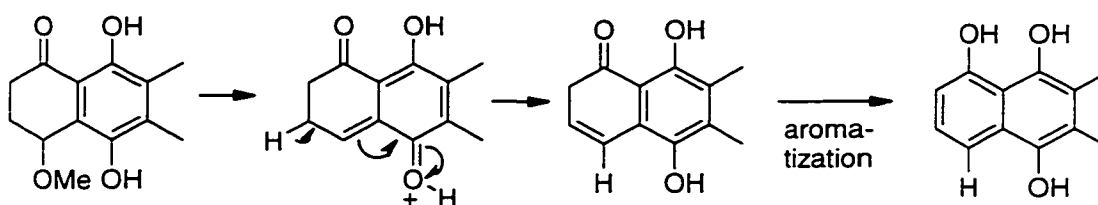
In this case the Diels-Alder reaction was predicted to be regioselective as shown on the basis of Molecular Orbital calculations and Frontier Molecular Orbital analysis. Other laboratories have demonstrated that regioselectivity of such reactions can be controlled through the use of halogen substituents (Scheme 43).<sup>14</sup>

Scheme 43



In the present case, the Diels-Alder reaction was expected to provide the four carbon atoms to complete the A-ring as well as functional groups which could act as the precursors to the keto and methoxy groups present in cysfluoretin. In the synthetic design it was important that the method for introducing these groups be relatively mild since elimination followed by enolization might readily convert the system into an aromatic ring (Scheme 44).

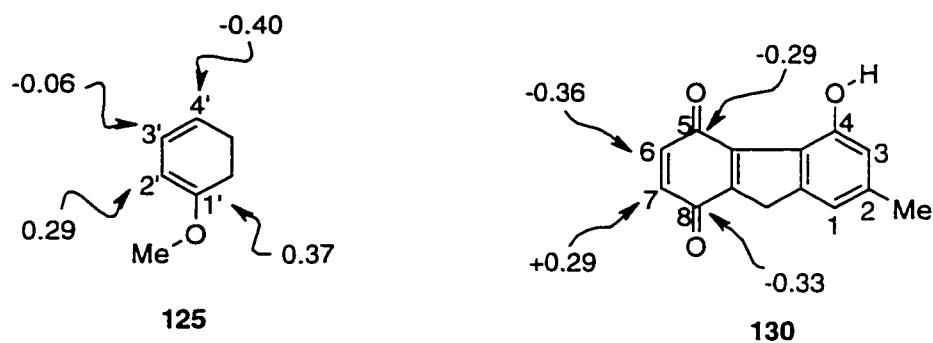
**Scheme 44**



It was felt that the expected adduct **118** could be aromatized to the hydroquinone and appropriately O-protected to give **119**. Oxidative cleavage of the olefinic linkage in **119** gives the diketone **120**. Baeyer-Villiger reaction would yield **121** and acid catalyzed acetal cleavage should give **122**. Borohydride reduction of the ketone **122** and O-methylation should give **123**. Hydrolysis of the acetate and oxidation (e.g. PCC) of the alcohol should generate ring A of cysfluoretin.

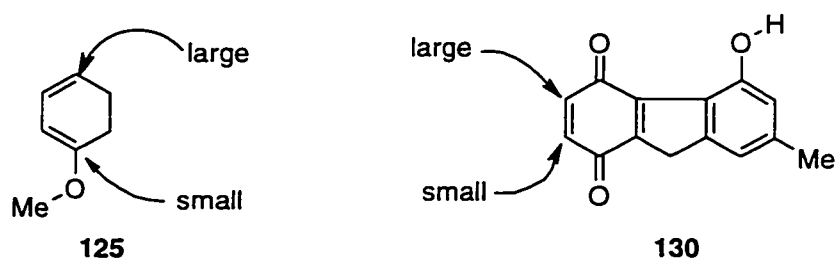
Semi-empirical molecular orbital calculations using the PM3 Hamiltonian were carried out for 1-methoxy-1,3-cyclohexadiene **125** and for the quinone **130** (Table 6).

Figure 28

Table 6- Semi-empirical Orbital Calculations of **125** and **130** Using PM3 Hamiltonian

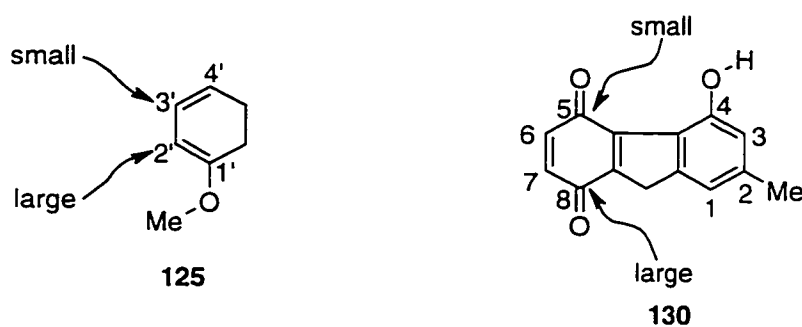
HOMO (-10.94 e.v.)		LUMO (-10.94 e.v.)	
Atom Position	Coefficient	Atom Position	Coefficient
4'	-0.40	5	-0.29
3'	-0.06	6	-0.36
2'	+0.29	7	+0.29
1'	+0.37	8	-0.33

Figure 29 Primary orbital interaction

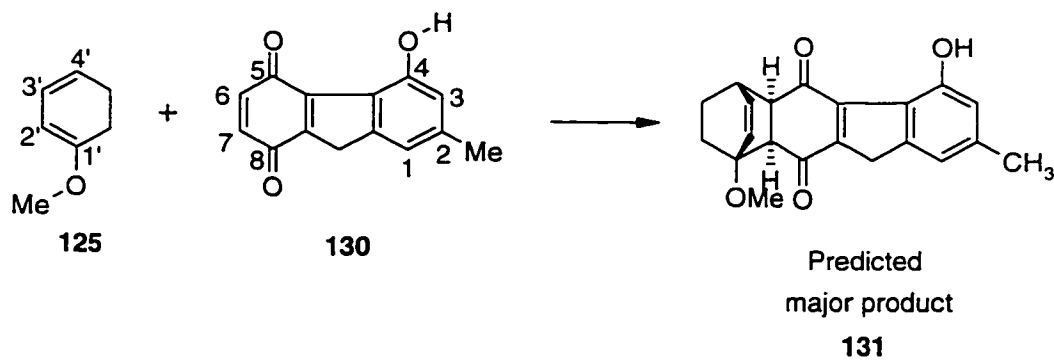


Frontier Orbital analysis<sup>70</sup> employing the HOMO coefficients for the diene **125** and the LUMO coefficients for the quinone **130** predict that the adduct **131** should be preferred based on the preferred interaction of the atom with the larger HOMO coefficient at the terminus of the diene (C<sub>4'</sub> in this case) with the atom with the larger LUMO coefficient at the terminus of the dienophile (C<sub>6</sub> in **130**) (i.e. large<sub>diene</sub>-large<sub>dienophile</sub> and small<sub>diene</sub>-small<sub>dienophile</sub> combination) (Figure 29). The same prediction is obtained from a secondary orbital interaction analysis in which the interaction of the C<sub>2'</sub> of the diene with C<sub>8</sub> of the dienophile and C<sub>4'</sub> of the diene with C<sub>5</sub> of the dienophile is preferred (Figure 30).

**Figure 30** Secondary Orbital Interaction



**Scheme 45**



Before preparing the substituted cyclohexadiene **117** and exploring control of the regioselectivity of the cycloaddition process, it was decided that some simple probes of the cycloaddition/oxidation sequence **116**→ **118**→ **120** should be carried out. Thus, the commercially available 1-methoxy-1,3-cyclohexadiene **125** was reacted with naphthoquinone **132** (Scheme 46). The reaction proceeded readily at room temperature to give the adduct **133** in 60% yield. The adduct exhibited a molecular ion (M+1) at  $m/z$  269 in CI-NH<sub>3</sub> as expected for the molecular formula C<sub>17</sub>H<sub>16</sub>O<sub>3</sub>. As expected, the <sup>13</sup>C NMR spectrum of the adduct contained 17 peaks. Signals corresponding to the two keto groups of the adduct appear at  $\delta$  195.6 and 197.6 ppm. Signals at 24.6 ppm and 28.8 ppm are assignable to the -CH<sub>2</sub>-CH<sub>2</sub>- bridge carbons and a signal at  $\delta$  36.6 ppm is assignable to the bridgehead carbon C<sub>6</sub>. A signal at 79.7 ppm likely corresponds to the bridgehead carbon 3. The signals appearing at 51.1, 51.7, and 52.2 are assigned to the carbon 2, 7, and 17. The vinyl carbons 4 and 5 likely give rise to signals at 126.1 and 126.8 ppm. In addition, there are six signals in the  $\delta$  131.0 to  $\delta$  137.4 range which correspond to the aromatic carbons.

Scheme 46

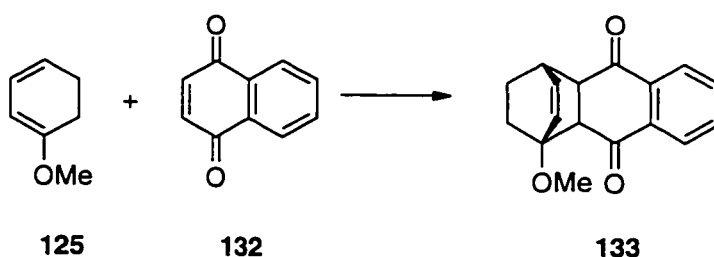
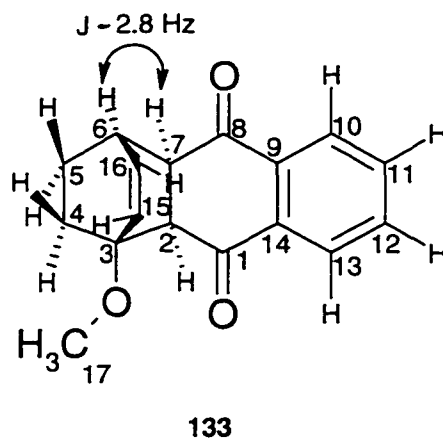


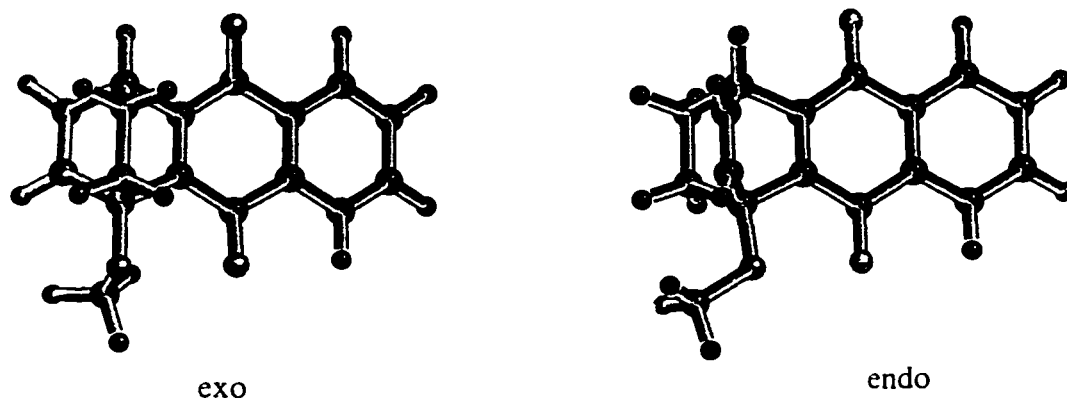
Figure 31



In the  $^1\text{H}$  NMR spectrum (250 MHz), a 2H multiplet at  $\delta$  7.80-7.89 ppm corresponding to the hydrogens ortho to the keto groups and a 2H multiplet  $\delta$  7.58-7.68 corresponding to the remaining aromatic hydrogens are observed. The two vinyl hydrogens give rise to a multiplet at  $\delta$  5.89-5.99 ppm.

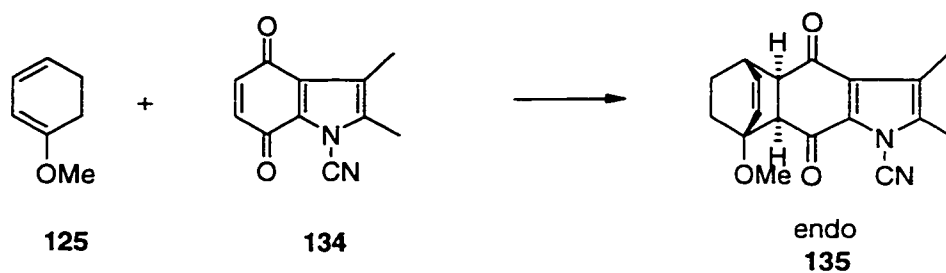
The  $-\text{CH}_2-\text{CH}_2-$  bridge hydrogens give rise to a 2H multiplet in the  $\delta$  1.41-1.59 ppm range and another 2H multiplet in the 1.85-2.05 ppm range. A narrow multiplet at  $\delta$  3.1 is assignable to the bridgehead hydrogens at  $\text{C}_6$ . The hydrogen at  $\text{C}_2$  appears as a doublet ( $J_{2,7} = 8.9$  Hz) at 3.54 ppm and the signal for the hydrogen at  $\text{C}_7$  appears as a doublet of doublets ( $J = 8.9, 2.8$  Hz) at 3.32 ppm. The methoxy group appears as a 3H singlet at  $\delta$  3.47 ppm. An I.R. band at  $\nu = 1680\text{ cm}^{-1}$  agrees with the diketone structure.

**Figure 32 Model of the exo and endo adducts generated by a Molecular Mechanism**  
Energy minimization using the Tripos forcefield in Sybyl 6.3®



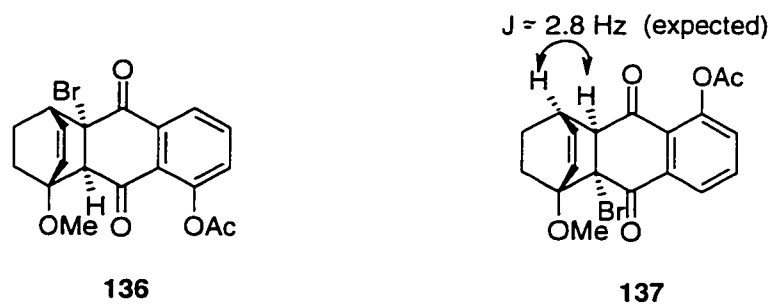
The stereochemistry of the adduct is likely endo as shown in Figures 31 and 32. Examination of models of the possible exo and endo adducts reveals the dihedral angle H<sub>7</sub>-C<sub>7</sub>-C<sub>6</sub>-H<sub>6</sub> to be approximately 60° for either adduct so that the Karplus rule is not useful here in determining the relative stereochemistry. However, previous work in this laboratory has shown, through X-ray crystallographic analyses, that **125** and **134** react to give an endo adduct **135** exclusively. We therefore believe that the single adduct found in this study is also endo.<sup>71</sup>

Scheme 47

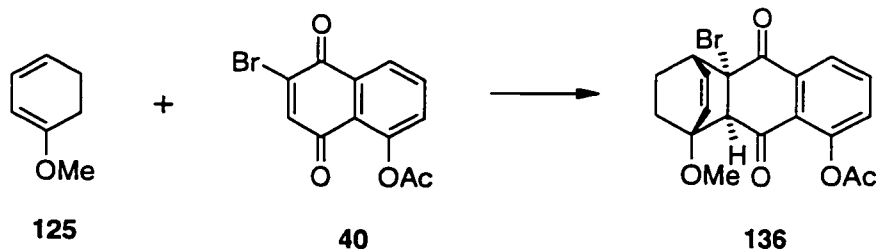


The Diels-Alder reaction between 1-methoxy-1,3-cyclohexadiene **125** and 2-bromo-5-acetoxynaphthoquinone **40** was also examined briefly (Scheme 48). The major product (53% yield) was found to be the adduct **136**.

Figure 33



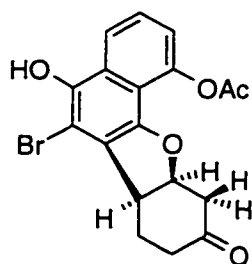
Scheme 48



From the  $^1\text{H}$  NMR spectrum (250 MHz), the 3 aromatic hydrogens of the benzyl ring appear respectively as a doublet ( $\delta$  7.84,  $J$ = 8.0 Hz), a triplet ( $\delta$  7.70,  $J$ = 7.6 Hz), and a doublet ( $\delta$  7.31,  $J$ = 8.0 Hz). One vinyl hydrogen gives rise to an apparent doublet at  $\delta$  6.37 ppm ( $J$ = 8.3 Hz) and the other to a triplet 6.18 ( $J$ = 7.8 Hz). The four  $-\text{CH}_2-\text{CH}_2-$  bridge hydrogens give rise to four different multiplets in the following range;  $\delta$  1.50-1.56 ppm, 1.75-1.85 ppm, 1.96- 2.05 ppm, and 2.22- 2.34 ppm. A narrow multiplet at  $\delta$  3.15 is assignable to the bridgehead hydrogens at  $\text{C}_6$ . The hydrogen at  $\text{C}_7$  appears as a singlet at 3.60 ppm consistent with structure **136** in which the  $\text{C}_7\text{-H}$  has no hydrogen on an adjacent carbon and not consistent with structure **137** for which a coupling constant of approximately 2.8 Hz is expected by analogy with structure **133**. The methoxy group occurs as a 3H singlet at  $\delta$  3.54 ppm as does the acetoxy group at  $\delta$  2.36 ppm. The I.R. band at  $\nu= 1760\text{ cm}^{-1}$  also agrees with the diketone structure.

An interesting minor product **138** (~5%) was also isolated and did not have the spectroscopic characteristics of a simple Diels-Alder adduct. By observing the  $^1\text{H}$  NMR

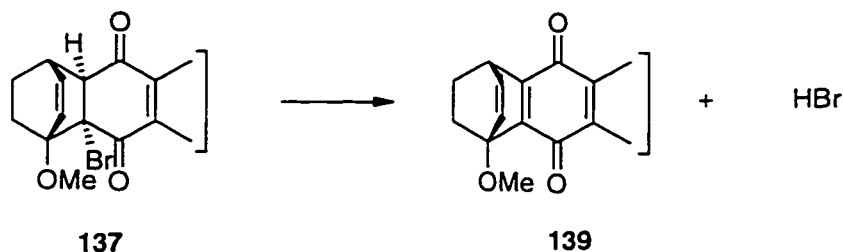
**Figure 34**



**138**

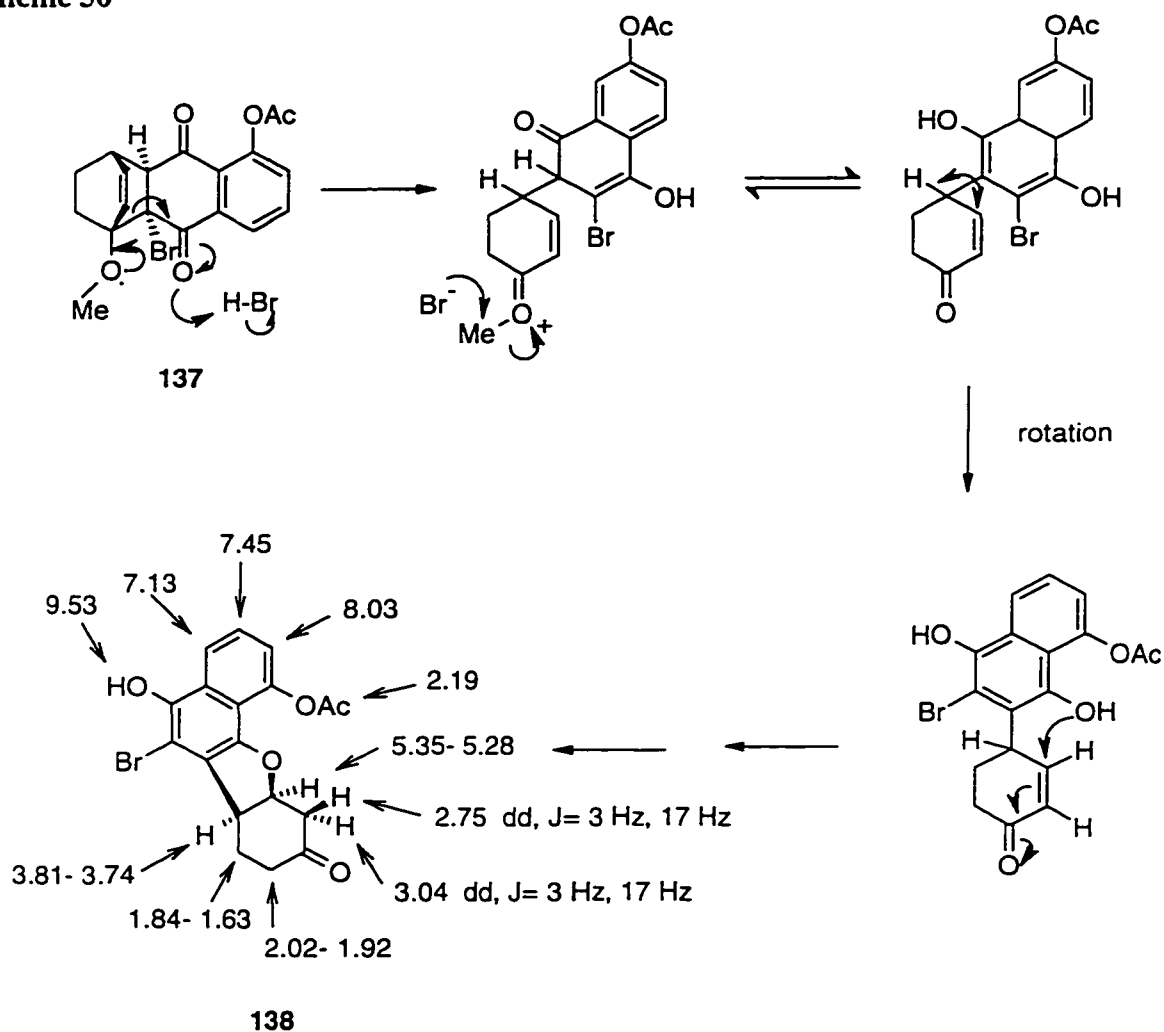
spectrum of the adduct, it is noticed, among other things, that no vinyl hydrogen are present and that the methoxy group has disappeared. A review of the literature describing the original studies by Birch and coworkers<sup>72</sup> revealed that the Diels-Alder adducts between methoxy cyclohexadiene are sensitive to acid and can undergo a C-C bond cleavage reaction adjacent to the methoxy group. In the present example, the presence of a bromine atom in the adduct creates the possibility that HBr elimination, to generate the quinone **139**, might lead to the release of sufficient acid to catalyze the decomposition of some of the adduct. A possible acid catalyzed process is shown in Scheme 50.

Scheme 49



The structure **138** is in agreement with the <sup>1</sup>H NMR spectrum as assigned in Scheme 50. Although this structural assignment is based on incomplete spectroscopic data, the fact that this compound represents a minor component of the product led us to not pursue this structural characterization further. If the structure **138** is correct this would mean that the major adduct is **136** and the minor adduct **137** which rearranged to **138**.

Scheme 50

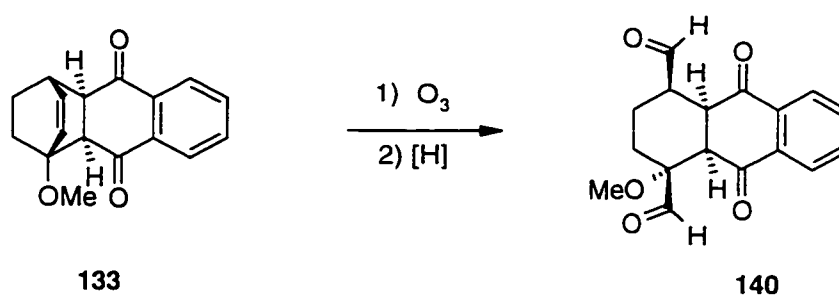


#### 4.2 Oxidative Cleavage of the Olefin to Aldehydes

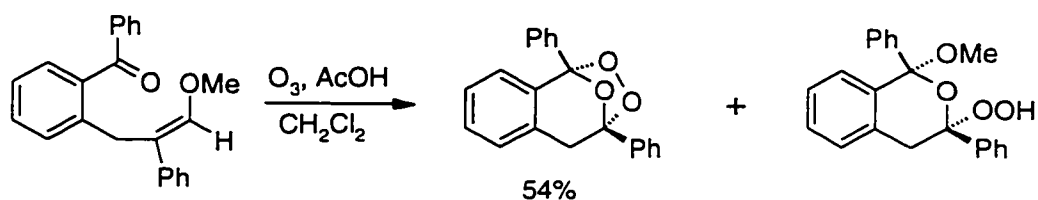
The next stage of the process involved oxidative cleavage of the  $\text{C}=\text{C}$  bond which we expected could be carried out readily through ozonolysis. In practice, however, numerous attempts at ozonolysis with various workup procedures such as  $\text{Et}_3\text{N}$ <sup>73</sup> or  $\text{NaI}$   $\text{AcOH}/\text{Na}_2\text{S}_2\text{O}_3$ ,<sup>74</sup> resulted in extensive decomposition. The presence of a weak aldehyde peak in the  $^1\text{H-NMR}$  of the decomposition mixture was noted. The failure in the ozonolysis

of the adduct **133** to yield the desired dialdehyde **140** cleanly (Scheme 51) led us to examine the literature for possible reasons for the formation of a complex mixture of products. We found that anomalous results in ozonolysis reaction were observed by McCullough et al.<sup>75</sup> in ozonolyses of alkenes possessing keto groups or keto precursor groups near the C=C bond which was being cleaved (scheme 52). In such cases the keto group was found to trap the carbonyl oxide intermediate to form an unusual bicyclic ozonide product which was isolated in some cases but which decomposed to other products in other cases.

Scheme 51

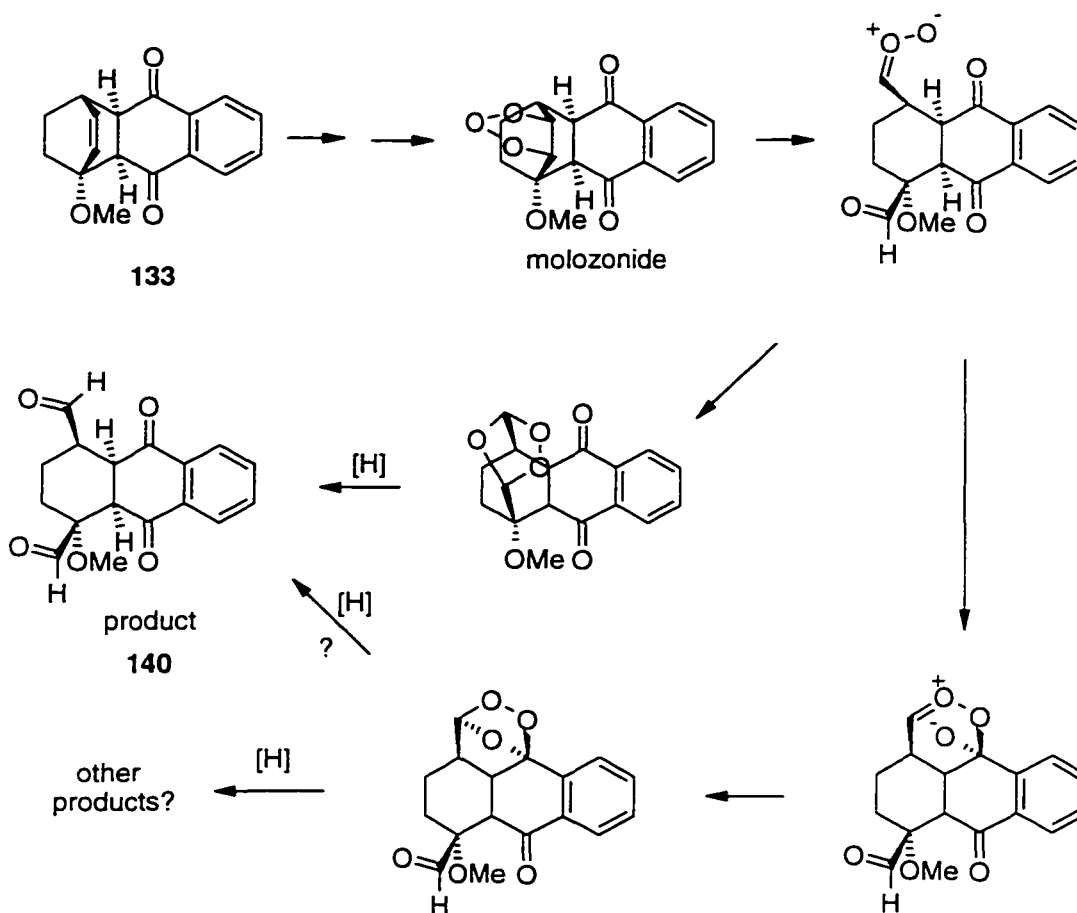


Scheme 52



In the present case the alkene under study also possesses keto groups which might lead to anomalous ozonide formation as shown below.

Scheme 53



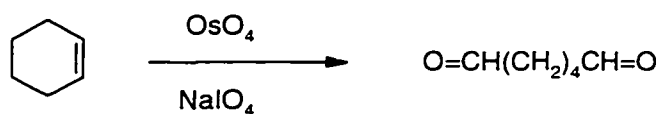
Although examination of the molecular models suggests that such a product is possible, it is not clear why such a species would not simply undergo reduction to the desired product in the workup.

Other reports in the literature suggest that the use of aprotic solvents in ozonolysis reactions can sometimes result in the formation of polymeric products and that better results are obtained when protic solvents, such as acetic acid, are added.<sup>75, 76</sup> In our study the

ozonolyses were done in  $\text{CH}_2\text{Cl}_2$ . Thus, it would seem logical to propose that further ozonolysis attempts be carried out with protic solvents present. Also, it might be useful to modify or protect the keto groups which are near the  $\text{C}=\text{C}$  bond to be cleaved. Aromatization of the diketone prior to ozonolysis might be desirable (as in Scheme 40).

Since other aspects of this project were showing more promise this approach to cysfluoretin was not pursued further. It is suggested, for future reference, that the oxidative cleavage of the  $\text{C}=\text{C}$  bond be performed as described above or under milder ozonolysis conditions by adding pyridine to the reaction.<sup>77</sup> Methods other than ozonolysis, which may be causing oxidative transformation of the aromatic ring system **133** and polymerization, could be explored. For example, useful  $\text{C}=\text{C}$  bond cleavage has been achieved using  $\text{OsO}_4/\text{NaIO}_4$ <sup>78, 79</sup> as shown in Scheme 54 below.

**Scheme 54**



## Chapter 5: Preliminary Results Concerning Inhibition of HIV-1 RT by Quinones Prepared in this Study

As indicated in the introduction, one of the goals of this study was to produce substituted naphthoquinones specifically for the purpose of exploring the effects of quinone structure on inhibition of HIV-1 RT. Very recently preliminary testing of some of the synthetic quinones described above has been carried out at the M<sup>c</sup>Gill AIDS Centre and some comments concerning these results are described below.

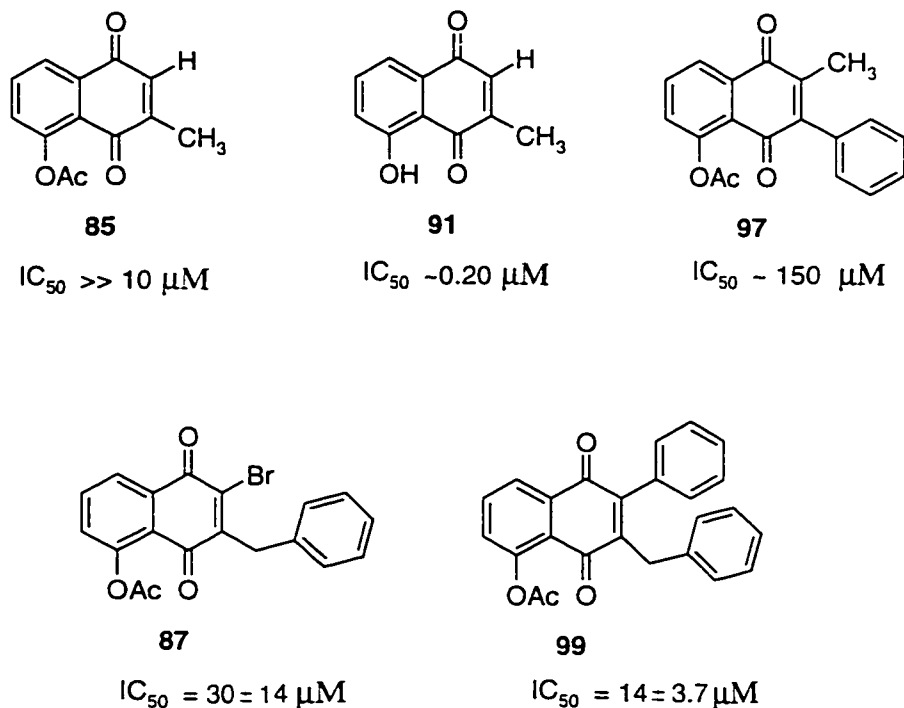
In the earlier work carried out by Take at al.<sup>8</sup> (Table 1, p 28), 1,4-naphthoquinone **25** and 5-hydroxy-1,4-naphthoquinone **26** were found to be comparable in activity against HIV-1 RT. RNA-dependent polymerase activity as measured, with poly (rA)/ oligo (dT) as template/primer (92% and 95% inhibition respectively at 50 µg/mL). Other substitutions were found to decrease activity.

For example 2,3-dichloro-1,4-naphthoquinone **27** exhibited 85% inhibition under the same conditions and 2-methyl-1,4-naphthoquinone **28** exhibited 70% inhibition. Interestingly 2-ethyl-1,4-naphthoquinone **29** was reported to be devoid of activity against HIV-1 RT although it retained activity against the RT from AMV and some activity against DNA polymerases.<sup>8</sup> In addition compounds such as **30-32** in which there is a C-2 hydroxyl group were all reported to be inactive against HIV-1 RT as well as AMV- RT and only weakly active against DNA polymerases.

The preliminary biochemical data for the compounds prepared in this study were obtained by Dr. John Barnard at the Lady Davis Institute for Medical Research and the M<sup>c</sup>Gill AIDS Research Centre using somewhat different conditions than those employed in the earlier study by Take et al.<sup>8</sup>. The enzyme employed in the current study was HIV-1 RT created by coexpression of the p66 and p51 subunits of RT in a recombinant strain of *E. coli*. The earlier work reported the use of RT generated by expression in *E. coli* of the RT gene which likely yielded mostly the p66 homodimeric form of RT.

In addition, the assay procedure in the present study employed poly rC/ oligo dG (18-20) as the template/ primer whereas the older work employed poly (rA)/ oligo dT (12-18). As a result, direct comparison of the results must be done with caution.

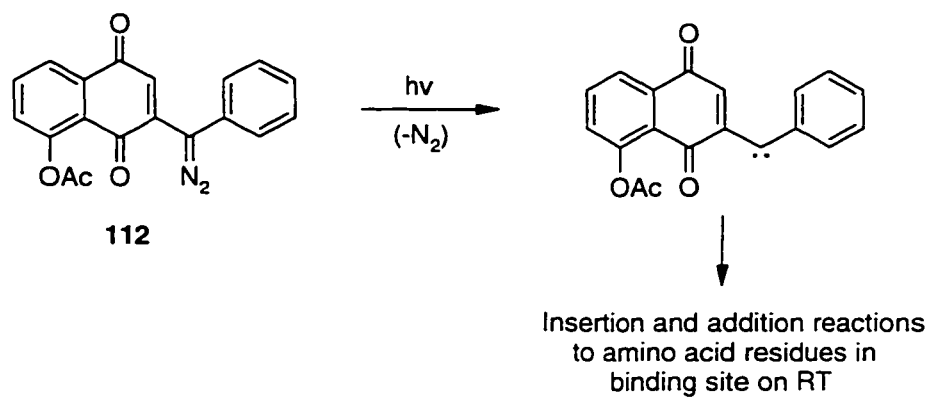
In any event, the present results suggest that hydroxyl substitution at C<sub>5</sub> is very beneficial with respect to inhibitory potency. Thus **91** exhibits an IC<sub>50</sub> of 200 nM whereas the IC<sub>50</sub> for **85** is well in excess of 10 μM. These preliminary data also clearly indicate that the quinone binding site on HIV-1 RT can tolerate a significant amount of steric bulk in the quinone inhibitor as exhibited by the C<sub>3</sub>-benzyl derivatives **87** (IC<sub>50</sub>= 30 ± 14 μM) and **99** (IC<sub>50</sub>= 14 ± 3.7 μM). Comparison of the good activity of **99** with the much poorer activity of **85** (>> IC<sub>50</sub> 10 μM) where R<sub>3</sub>= CH<sub>3</sub> suggests that the phenyl ring of the benzyl group interacts in a beneficial way with the binding site.



The activity of the deacetylated versions of **87**, **97**, **99** are predicted to be much improved over those of the acetate and such compounds should be prepared and analyzed along with the other quinones prepared in this study.

It should also be pointed out that the diazo compound **112** prepared in this study and/or the corresponding phenol should also be analyzed for inhibition of RT particularly since the diazo group might serve as a photoaffinity binding site for the quinone binding pocket. That is, if **112** proves to be a good reversible inhibitor of RT in the dark, then photolysis of a

solution of **112** on RT might lead to irreversible covalent modification of the binding site on the protein and eventually to an identification of this binding site on complex RT protein.



In any event, the preliminary biochemical data suggest that more extensive explorations of the interactions of substituted quinones with RT are in order and it seems likely that the synthetic methods developed in this study will play a useful role in generating such potential inhibitors of HIV-1 RT.

## Chapter 6: Conclusion

The regioselective synthesis of disubstituted naphthoquinone derivatives using the Jacobsen-Thorsell reaction in concert with hydrogenolysis has been developed. The disubstituted naphthoquinones generated in this study, as well as future derivatives, can have useful applications in several fields of chemistry either as precursors to more complex molecules (as in the cases of the synthesis of analogs of the kinamycins), or as inhibitors of the HIV-1 RT in the fight against AIDS.

Among the different quinone derivatives generated in this study, compound **99** seems to be a promising lead in the development of more potent quinone inhibitors of the HIV-1 RT. As indicated in this study, the presence of a hydroxyl group at C-5 seems to remarkably increase the activity.

As well, the possibility that the regioselective stepwise alkylation of naphthoquinones described in this thesis may be applied in an unambiguous route to prekinamycin has been explained. The difficulties observed in previous methods found in the literature concerning the C-11 diazo transfer on a benzo[b]fluorenone ring system may be avoided as mentioned in Chapter 3. The model diazo transfer method examined in the present work suggest that no major problem should prevent the incorporation of the diazo group in the proposed precursor **110** toward the synthesis of prekinamycin.

Studies regarding the regioselective synthesis of the oxygen functionalities present in the D ring of kinamycins were carried out using a simpler model. The regioselective acylation of the epoxy-diol **107** by acetate to give **141** was unsuccessful, as was the ring opening using potassium acetate or water.

Preliminary studies concerning the synthesis of analogues of cysfluoretin have been performed. It has been proposed that the B, C, and D ring system of cysfluoretin might be generated from the quinone **116** (Scheme 40) and that the A ring could be added via a Diels-Alder reaction with **117** as the diene. Frontier Orbital analysis employing the HOMO coefficients for the 1-methoxy-1,3-cyclohexadiene (**125**) and the LUMO coefficients for the quinone **130** predict that this reaction should afford the desired regioselectivity. In a model study the adduct **133** was formed from **125** and 1,4-naphthoquinone. Oxidative cleavage of the olefinic linkage in **133** did not afford the desired diketone **140**. It has been suggested that different or milder conditions might afford better results.

## Chapter 7: Experimental

### 7.1 General procedures

All reagents employed were purchased from the Aldrich Chemical Company, Inc and all reactions were performed under a nitrogen atmosphere, unless otherwise noted. Each reaction was monitored by thin layer chromatography using aluminium-backed sheets precoated with silica gel (0.2 mm) using an ultraviolet lamp (254 nm), or by  $^1\text{H}$  NMR spectroscopy. Column chromatography was accomplished using silica gel (70-230 mesh A.T.S.M., EE. Merck).

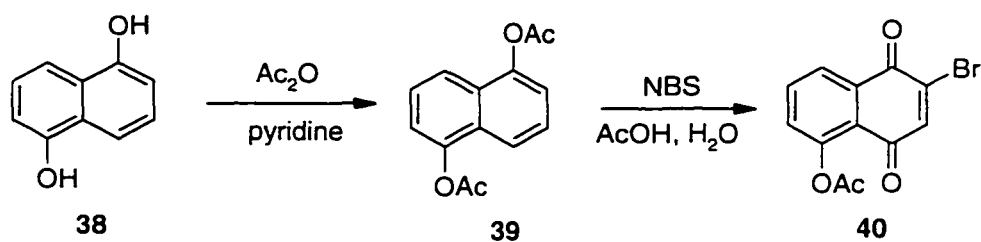
Infrared spectra were performed on an MB-100 Fourier Transform spectrometer using NaCl discs. The peaks of interest are given in  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were performed on a Brüker AC-250 with deuteriochloroform as the solvent unless otherwise indicated. As internal standard, tetramethylsilane was used for  $^1\text{H}$  NMR and deuteriochloroform (centered at 77.0 ppm) was used for  $^{13}\text{C}$  NMR, unless otherwise noted. All  $^{13}\text{C}$  NMR spectra were broadband decoupled. The spectral parameters are listed in the following order: (frequency, solvent) chemical shifts in ppm. Nuclear Overhauser effect (nOe) experiments were performed by Ms. Jan Venne on a Brüker AMX-500 NMR spectrometer.

Low and high resolution mass spectra were recorded at the McMaster Regional Center for Mass Spectrometry and elemental analyses were recorded by M-H-W Laboratories in Arizona.

## 7.2 Regioselective Synthesis of 5-Acetoxy-1,4-naphthoquinones Derivatives

### Preparation of 5-Acetoxy-2-bromo-1,4-naphthoquinone **40**



1,5-Diacetoxynaphthalene **39** was prepared from 1,5-dihydroxynaphthalene **38** as described by S. Mithani.<sup>5</sup> 2-Bromo-5-acetoxynaphthoquinone was prepared from **40**, also as described by Mithani,<sup>5</sup> and was found to be identical to an authentic sample by TLC and  $^1\text{H}$  NMR analysis.

## Jacobsen-Thorssell Alkylation/Arylation of 5-Acetoxy-2-bromo-1,4-naphthoquinone: General Procedure Using a High Acetonitrile to Water Ratio

The Jacobsen-Thorssell alkylation/arylation of 5-acetoxy-2-bromo-1,4-naphthoquinone using high acetonitrile to water ratio were performed initially as described by Mithani.<sup>5</sup> The reactions described in Table 2 (p. 48) all have in common the fact that acetonitrile was the sole solvent before the addition of the aqueous ammonium persulfate solution. Any deviations from the general procedure stated below are described in Table 2.

In a 2-neck round bottomed flask, the quinone **40** (0.525 g; 1.78 mmol) was dissolved in acetonitrile (15.0 mL). The carboxylic acid (1.78 mmol) and the silver nitrate (0.562 g; 0.481 mmol) were added. An addition funnel containing an aqueous ammonium persulfate solution ( 1.06 g; 4.63 mmol dissolved in water (18.0 mL)) with a septum at the top was installed on one neck. A condenser hooked to a nitrogen line was mounted on the second neck. The system was flushed with argon from a balloon attached via a needle through the septum in order to remove any oxygen from the system. The reaction mixture was then warmed to 60°C, with stirring. The aqueous ammonium persulfate solution was added slowly. After the addition of all the persulfate, the mixture was heated to 95 °C and the reaction was monitored by TLC until the reaction was thought to be complete. The reaction was then cooled to room temperature. Water was added (~ 150 mL) and the mixture was extracted with methylene chloride (~6 x 30 mL or until the organic extract was colourless). The organic extracts were combined, dried over sodium sulfate and the solvent was removed *in vacuo*. A <sup>1</sup>H NMR spectrum of the crude product was obtained. The

reaction mixture was then dissolved in a minimal amount of ethyl acetate and this solution was used to preadsorb the sample on silica gel (6x w/w) which was applied to the top of a silica gel column. Elution with 30% ethyl acetate/hexane solution was used to purify the product. The results of the reactions are described in Table 2 (p 48).

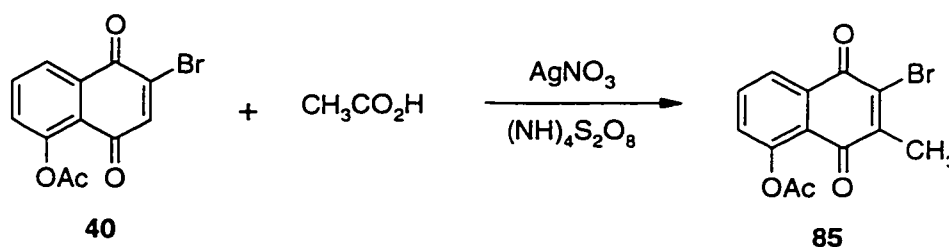
### **General Procedure: Using a Lower Acetonitrile to Water Ratio**

The Jacobsen-Thorssell alkylation/arylation of 5-acetoxy-2-bromo-1,4-naphthoquinone **40** using lower acetonitrile to water ratio differs from the procedure mentioned above in the fact that the solvent prior to the addition of the aqueous ammonium persulfate is not solely acetonitrile but a 3:1 mixture of water/acetonitrile (see Table 3 (p. 51) for reaction conditions).

In a 2-neck round bottomed flask, the quinone **40** (0.500 g; 1.69 mmol) was dissolved in acetonitrile ( 2.1 mL) and water was added (6.3 mL). The carboxylic acid (2.96 mmol) and the silver nitrate (0.086 g; 0.51 mmol) were then added. A septum was installed on one neck and a condenser, hooked to a nitrogen line, was mounted on the second neck. The system was flushed with argon (balloon with needle through the septum) to remove any oxygen from the system. The reaction mixture was then warmed to 60°C, under stirring. The aqueous ammonium persulfate solution (0.424 g; 1.86 mmol dissolved in water (1.7 mL)) was added in small aliquots (0.1 mL every 10-15 min) with the aid of a syringe through the septum. After the addition of all of the persulfate solution, the reaction was kept at 60°C for 10 min. The reaction was then cooled to room temperature. Water was added (~ 150 mL) and extractions with methylene chloride were performed (~6 x 30 mL or until the organic extract

became colourless). The organic extracts were combined, dried over sodium sulfate and the solvent was removed *in vacuo*. A  $^1\text{H}$  NMR spectrum of the crude product was obtained. The reaction mixture was then dissolved in a minimal amount of ethyl acetate and this solution was used to preadsorb the sample on silica gel (6x w/w) which was applied to the top of a silica gel column. Elution with 30% ethyl acetate/ hexane solution was used to purify the product. The results of the reactions are described in Table 3 (p. 51). In cases where the reaction was found to be incomplete by  $^1\text{H}$  NMR analysis, the reaction product was subjected to the reaction conditions again to ensure complete consumption of the starting material which was difficult to separate from the product. More details of such “recycling” experiments are presented in Table 3 (p 51). In each case below, the product was compared to an authentic sample by TLC and  $^1\text{H}$  NMR analysis.

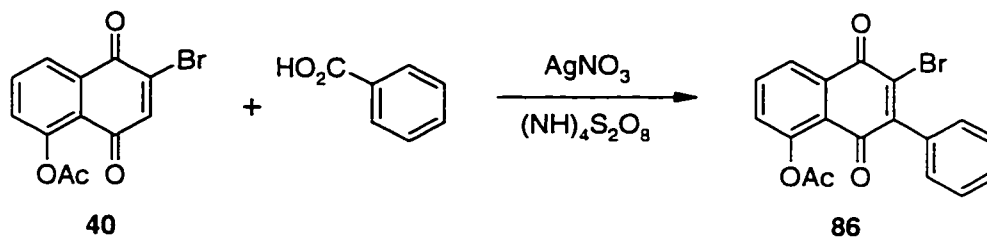
#### Synthesis of 5-Acetoxy-2-bromo-3-methyl-1,4-naphthoquinone 85



Yield: 87% (See Table 3)

$^1\text{H}$  NMR (250MHz,  $\text{CDCl}_3$ )  $\delta$  8.11 (d,  $J=7.5$  Hz, 1H), 7.73 (t,  $J=7.9$  Hz, 1H), 7.38 (d,  $J=7.9$  Hz, 1H), 2.45 (s, 3H), 2.34 (s, 3H)

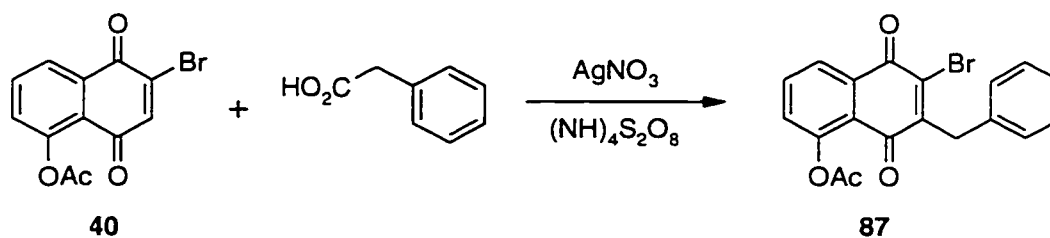
### Synthesis of 5-Acetoxy-2-bromo-3-phenyl-1,4-naphthoquinone 86



Yield: 72% (See Table 3)

$^1\text{H NMR}$  (250MHz,  $\text{CDCl}_3$ )  $\delta$  8.21 (dd,  $J=7.8, 1.2$  Hz, 1H), 7.79 (t,  $J=7.9$  Hz, 1H), 7.51-7.42 (m, 4H), 7.31-7.27(m, 2H), 2.38 (s, 3H)

### Synthesis of 5-Acetoxy-3-benzyl-2-bromo-1,4-naphthoquinone 87



Yield: 96% (See Table 3)

$^1\text{H NMR}$  (250MHz,  $\text{CDCl}_3$ )  $\delta$  8.11 (dd,  $J=7.8, 1.2$  Hz, 1H), 7.73 (t,  $J=7.9$  Hz, 1H), 7.40-7.18 (m, 6H), 4.18 (s, 2H), 2.45 (s, 3H)

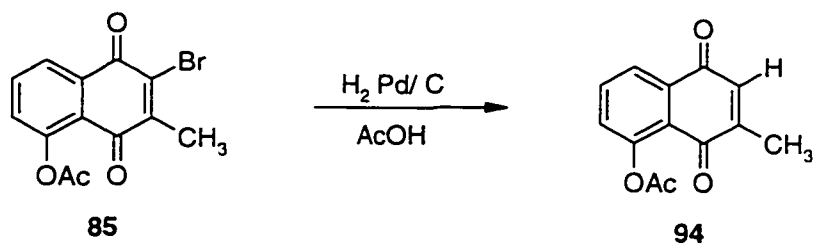
### Reductive Dehalogenation of 5-Acetoxy-2-bromo-3-substituted-1,4-naphthoquinone

The dehalogenation of the 2-bromo-3-substituted-naphthoquinones was performed using catalytic hydrogenation in a Parr hydrogenation apparatus. In a typical experiment, the quinone (3.88 mmol) was dissolved in acetic acid (150 mL), the solution was transferred to a 500 mL Parr hydrogenation bottle, and 2 equivalents of sodium acetate were added as well as about 0.40 g of 10% Pd/C. This reaction is believed to produce a substance poisoning the catalyst since when less catalyst was used, the reaction did not go to completion even after a week on the Parr apparatus. The Parr bottle was then set up on the Parr apparatus and the system was flushed 4 times with hydrogen by alternating evacuation with a water aspirator and pressurization from the H<sub>2</sub> reservoir in order to remove all traces of oxygen. The hydrogen pressure was set up at 50 psi and the reaction vessel was shaken for the appropriate length of time (see Table 4 page 57).

Shaking was stopped and the reaction vessel was closed off from the H<sub>2</sub> reservoir and evacuated with a water aspirator. The mixture was filtered through celite in order to remove the catalyst ensuring that the catalyst was always wet with solvent and not exposed to the air. The bottle and celite were rinsed with ethyl acetate. A TLC was run to show the presence of the hydroquinone and absence of starting material. The acetic acid/ethyl acetate solution containing the product was transferred to a round bottomed flask and the solution was cooled on an ice/salt bath (T is  $\approx -5$  °C). More EtOAc was added as needed to prevent freezing of the solution. DDQ (2 equiv.) dissolved in a minimal amount of acetic acid was then added, with vigorous stirring, to the cold solution over a period of about 30 min. The reaction was left for 1 h and the solvent was removed *in vacuo*. The flask was rinsed with several portions of

80% water/ methylene chloride in order to remove the product from it. Most of the reduced DDQ was now found in the aqueous phase. The organic phase was separated and washed several times with water and brine. The organic phase was then dried over sodium sulphate and the solvent removed *in vacuo*. Column chromatography on silica gel using 30% EtOAc/hexane as eluant gave the pure debrominated quinones. The yields and characterization data are summarized below.

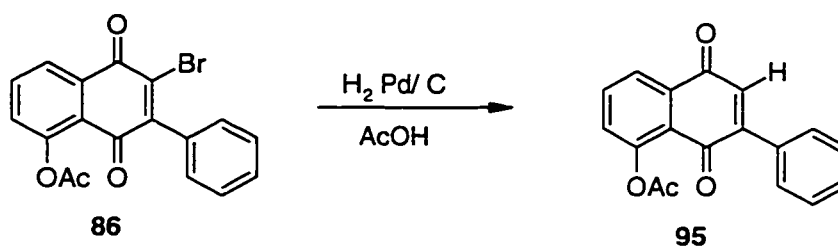
#### Synthesis of 5-Acetoxy-3-methyl-1,4-naphthoquinone 94



Yield:96%

<sup>1</sup>H NMR (250MHz, CDCl<sub>3</sub>) δ 8.02 (dd, J=7.7, 1.2 Hz, 1H), 7.73 (t, J=7.9 Hz, 1H), 7.35 (dd, J= 8.1, 1.2 Hz, 1H), 6.82 (q, J= 1.5 Hz, 1H), 2.46 (s, 3H), 2.14 (d, J=1.5 Hz, 3H); <sup>13</sup>C NMR (63MHz, CDCl<sub>3</sub>) δ 183.8, 183.6, 169.1, 148.9, 134.4, 133.7, 129.2, 124.3, 123.1, 20.8, 16.3; I.R. (cm<sup>-1</sup>) 1758, 1665.

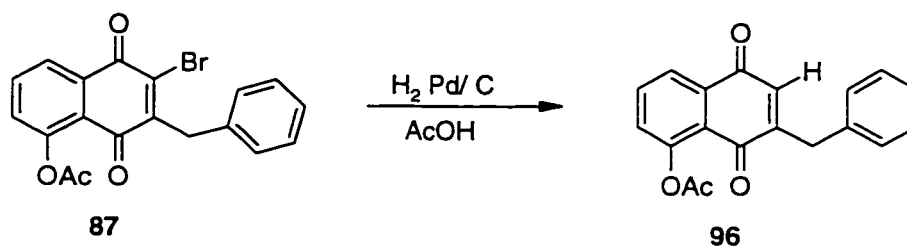
### Synthesis of 5-Acetoxy-3-phenyl-1,4-naphthoquinone 95



Yield: 84%

$^1\text{H}$  NMR (250MHz,  $\text{CDCl}_3$ )  $\delta$  8.09 (d,  $J=7.5$  Hz, 1H), 7.78 (t,  $J=7.9$  Hz, 1H), 7.48-7.39 (m, 6H), 7.03 (s, 1H), 2.45 (s, 3H);  $^{13}\text{C}$  NMR (63MHz,  $\text{CDCl}_3$ )  $\delta$  184.13, 183.2, 169.4, 149.7, 149.3, 134.6, 133.9, 133.7, 133.3, 130.0, 129.7, 129.4, 128.3, 124.5, 123.8, 21.1.

### Synthesis of 5-Acetoxy-3-benzyl-1,4-naphthoquinone 96



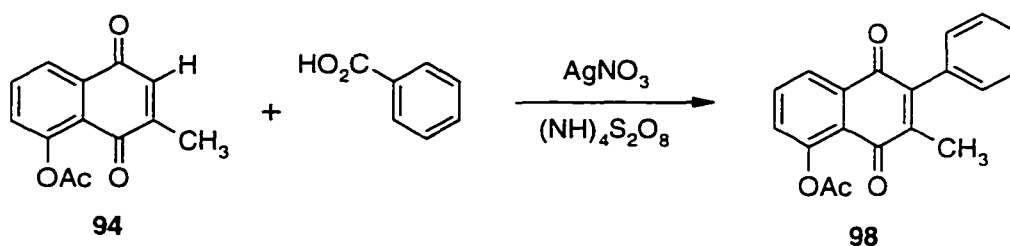
Yield: 95%

$^1\text{H}$  NMR (250MHz,  $\text{CDCl}_3$ )  $\delta$  8.01 (d,  $J=7.6$  Hz, 1H), 7.73 (t,  $J=7.9$  Hz, 1H), 7.38-7.18 (m, 6H), 6.49 (s, 1H), 3.84 (s, 2H), 2.48 (s, 3H);  $^{13}\text{C}$  NMR (63MHz,  $\text{CDCl}_3$ )  $\delta$  184.1, 183.5, 169.4, 152.0, 136.3, 134.6, 134.4, 133.7, 129.5, 128.8, 128.6, 128.4, 126.9, 124.6, 123.4, 35.4, 21.1; I.R. ( $\text{cm}^{-1}$ ) 1754, 1664; EIMS  $m/z$  (%) 306 (1), 264 (51)

### Jacobsen-Thorsell Alkylation/Arylation of 5-Acetoxy-3-substituted-1,4-naphthoquinone

The Jacobsen-Thorsell alkylation/arylation of 5-acetoxy-3-substituted-1,4-naphthoquinone followed essentially the same general procedure as the one explained when a 3:1 water/acetonitrile ratio was the initial solvent. The conditions for each reaction can be found in Table 5 (p 60).

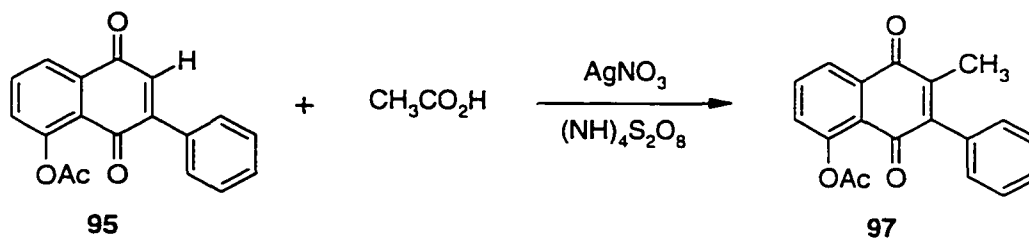
### Synthesis of 5-Acetoxy-3-methyl-2-phenyl-1,4-naphthoquinone 98



Yield: 33%

<sup>1</sup>H NMR (250MHz, CDCl<sub>3</sub>) δ 8.05 (dd, J=7.8, 1.1 Hz, 1H), 7.70 (t, J=7.9 Hz, 1H), 7.48-7.40 (m, 3H), 7.34 (dd, J= 8.0, 1.2 Hz, 1H), 7.21-7.17 9m, 2H), 2.46 (s, 3H), 2.02 (s, 3H); <sup>13</sup>C NMR (63MHz, CDCl<sub>3</sub>) δ 184.3, 183.4, 169.5, 145.4, 145.2, 134.5, 133.9, 133.3, 129.2, 128.5, 128.2, 125.1, 123.4, 21.1, 14.7; I.R. (cm<sup>-1</sup>) 1770, 1660; EIMS m/z (%) 306 (7), 264 (33), 249 (13); HRMS calcd for C<sub>19</sub>H<sub>14</sub>O<sub>4</sub> 306.0903, found 306.0892.

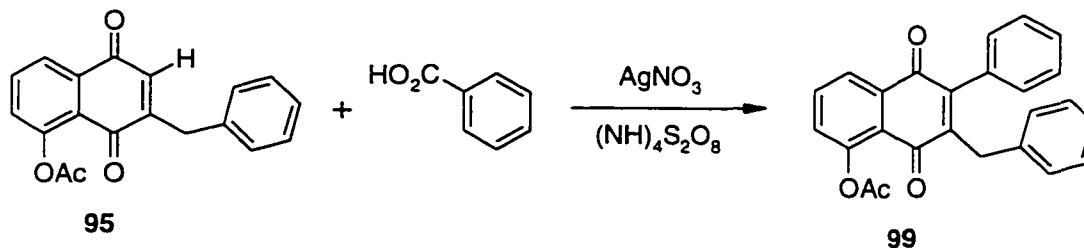
### Synthesis of 5-Acetoxy-2-methyl-3-phenyl-1,4-naphthoquinone 97



Yield: 46%

$^1\text{H NMR}$  (250MHz,  $\text{CDCl}_3$ )  $\delta$  8.13 (d,  $J=7.8$  Hz, 1H), 7.75 (t,  $J=8.0$  Hz, 1H), 7.47-7.36 (m, 4H), 7.20-7.17 (m, 2H), 2.38 (s, 3H), 2.03 (s, 3H);  $^{13}\text{C NMR}$  (63MHz,  $\text{CDCl}_3$ )  $\delta$  185.1, 182.8, 170.0, 149.4, 147.3, 143.1, 124.3, 133.9, 133.6, 129.3, 128.5, 128.2, 124.9, 123.5, 21.2, 14.4; I.R. ( $\text{cm}^{-1}$ ) 1763, 1660; EIMS  $m/z$  (%) 306 (20), 264 (45), 249 (7); HRMS calcd for  $\text{C}_{19}\text{H}_{14}\text{O}_4$  306.0887, found 306.0892.

### Synthesis of 5-Acetoxy-3-benzyl-2-phenyl-1,4-naphthoquinone 99

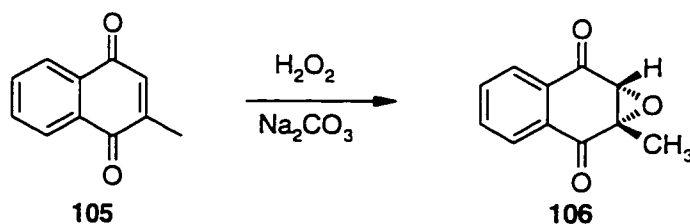


Yield: 93%

$^1\text{H}$  NMR (250MHz,  $\text{CDCl}_3$ )  $\delta$  8.06 (d,  $J=7.7$  Hz, 1H), 7.72 (t,  $J=7.9$  Hz, 1H), 7.43-7.35 (m, 4H), 7.21-7.12 (m, 5H), 6.94-6.91(m, 2H), 3.85 (s, 2H), 2.44 (s, 3H);  $^{13}\text{C}$  NMR (63MHz,  $\text{CDCl}_3$ )  $\delta$  183.9, 183.3, 169.5, 149.3, 146.1, 138.4, 134.6, 133.8, 133.0, 130.5, 129.5, 129.3, 128.6, 128.3, 128.2, 127.6, 126.2, 125.1, 123.6, 33.1, 21.1; I.R. ( $\text{cm}^{-1}$ ) 1772, 1662; EIMS  $m/z$  (%) 382 (1), 340 (25), 264 (20). As a result of the very low intensity of the molecule ion at 382 amu, a high resolution measurement was carried out on the  $(\text{M}-42)^+$  fragment HRMS  $(\text{M}-42)^+$  calcd. For  $\text{C}_{23}\text{H}_{16}\text{O}_3$  340.1109, found 340.1099. CIMS ( $\text{NH}_3$ )  $m/z$  (%) 400  $(\text{M}+\text{NH}_4)^+$  (48), 383  $(\text{M}+\text{H})^+$  (100).

### 7.3 Synthesis of Models of the Kinamycin Type Antitumour Antibiotics

#### Epoxidation of 2-Methyl-1,4-naphthoquinone **105**



A mixture of the quinone **105** (5.00 g; 26.6 mmol) in ethanol (20 mL) was stirred and heated to 65°C in order to effect complete dissolution of the quinone. The solution was cooled to 45°C, and an aqueous hydrogen peroxide solution (10 mL; 30%) was added followed by 2.5 g of sodium carbonate dissolved in 10 mL of water. Gas bubbles were

formed and the yellow color of the solution disappeared. A white foam appeared on top of the solution as well as on the side of the flask. After 5 min., the mixture was diluted with 250 mL of water and filtered. The fine powder which was collected was rinsed with water until completely white. The filtrate was slightly pink. The product was further dried in a dessicator under vacuum to yield a white powder ( 4.23 g; 78% yield) and was found to be homogeneous by TLC and  $^1\text{H}$  NMR analysis.

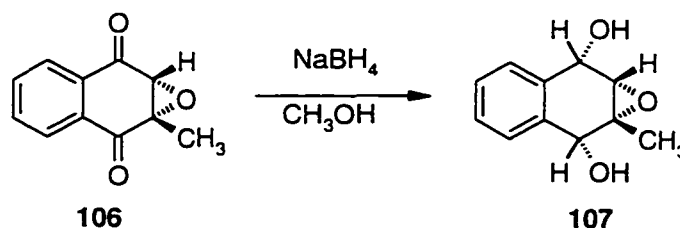
$^1\text{H}$  NMR (250MHz,  $\text{CDCl}_3$ ):  $\delta$  8.05-7.95 (m, 2H), 7.79-7.72 (m, 2H), 3.87 (s, 1H).

1.75 (s, 3H);  $^{13}\text{C}$  NMR (63MHz,  $\text{CDCl}_3$ ):  $\delta$  191.9 (overlap), 134.5, 134.3, 132.1, 132.0,

127.5, 126.8, 61.4, 14.7; I.R. ( $\text{cm}^{-1}$ ) 1686, 1190; EIMS  $m/z$  (%) 188 (33), 173 (29), 160

(22); HRMS calcd for  $\text{C}_{11}\text{H}_8\text{O}_3$  188.0459, found 188.0473.

### Reduction of 2,3-Epoxy-3-methyl-1,4-naphthoquinone **106**



A solution of 2,3-epoxy-3-methyl-1,4-naphthoquinone **106** ( 0.188 g; 1.0 mmol) was dissolved in 15 mL of methanol. The solution was stirred and cooled to  $0^\circ\text{C}$  with an ice bath. Sodium borohydride (0.038 g; 1.0 mmol) was added, the bath was removed, and the solution

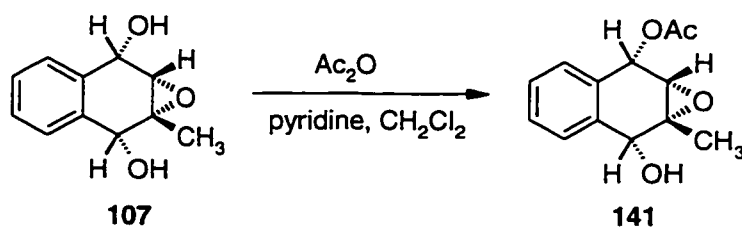
was stirred for 5 min. The bulk of the methanol was removed *in vacuo* and 125 mL of saturated ammonium chloride was added to quench the reaction. The reaction mixture was extracted with ethyl acetate (6 x 40 mL). The combined organic extracts were dried over sodium sulfate and the solvent was evaporated to yield the diol **107** as a white powder (0.191g; 99% yield). The product was found to be homogeneous by TLC and  $^1\text{H}$  NMR analysis.

$^1\text{H}$  NMR (250MHz, DMSO  $d_6$ ):  $\delta$  7.49-7.41 (m, 2H), 7.27-7.20 (m, 2H), 5.80-5.73 (m, 2H), 4.80 (d,  $J$ = 6.9 Hz, 1 H), 4.65 (d,  $J$ = 8.2 Hz, 1H), 3.33 (d,  $J$ = 1 Hz, 1H), 1.48 (s, 3H)

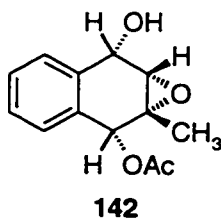
The two proton multiplet  $\delta$  5.80-5.73 disappeared upon addition of  $\text{D}_2\text{O}$  to the sample.

$^{13}\text{C}$  NMR (63MHz, DMSO  $d_6/\text{D}_2\text{O}$ ):  $\delta$  136.4, 135.7, 126.8, 125.5, 125.4, 68.7, 65.8, 61.9, 59.4, 19.2; I.R. ( $\text{cm}^{-1}$ ) 3257, 1046; EIMS  $m/z$  (%) 192 (23), 145 (40), 131 (100); HRMS calcd for  $\text{C}_{11}\text{H}_{12}\text{O}_3$  192.0770, found 192.0786.

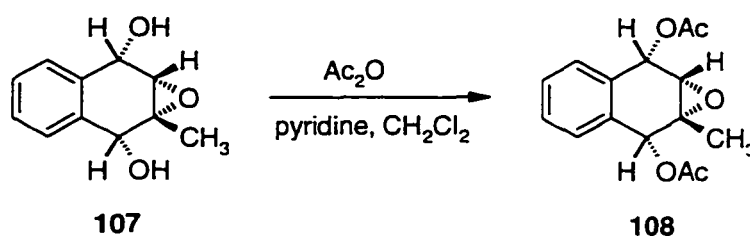
#### Attempted Monoacetylation of the 2,3-Epoxy-1,4-hydroxy-3-methyl-1,2,3,4-tetrahydronaphthalene **107**



A selective monoacetylation was tried by dissolving the epoxide **107** (0.100 g; 0.521 mmol) in methylene chloride (20 mL) and mixing it with 1 mole equivalent of acetic anhydride and 2 mole equivalents of pyridine at room temperature under nitrogen for 1 h. Dilute hydrochloride acid (5%) (~10 ml) was added to the solution to neutralize the remaining pyridine. Extraction of the reaction mixture was performed with ethyl acetate (6 x 10 mL), the combined organic extracts dried over sodium sulfate and the solvent evaporated. It has been determined, under the conditions used, that no major selection occurs. In the  $^1\text{H}$  NMR in  $\text{DMSO } d_6$ , four peaks were observed between  $\delta$  1.39 and 1.51 probably corresponding to the methyl groups in the four possible products shown in the scheme above: the starting material **107**, the two monoacetylated products **141** and **142**, and the diacetylated one **108**. Also, four peaks were observed between  $\delta$  2.24 and 2.30 corresponding most likely to the acetoxy peaks of the two monoacetylated and diacetylated products. In addition, four different peaks were also observed between  $\delta$  4.67 and 4.94, where the hydroxyl hydrogens would appear. The starting material would account for two of those peaks and each monoacetylated product for one each. Since only 1 equivalent of acetic anhydride was present, the reaction was stopped after its consumption.



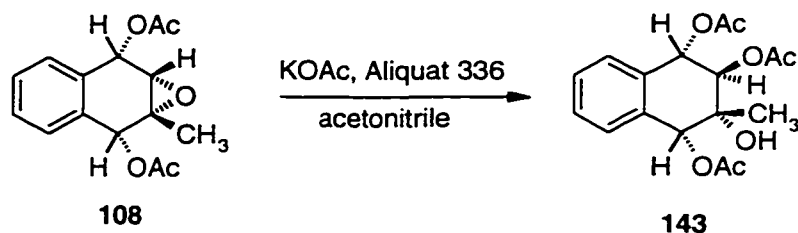
### Diacetylation of the 2,3-Epoxy-1,4-hydroxy-3-methyl-1,2,3,4-tetrahydronaphthalene 107



The product mixture obtained in the monoacetylation attempt described above was used for the diacetylation reaction. To the product mixture (0.060 g; ~ 0.3 mmol) dissolved in methylene chloride (15 mL) was added acetic anhydride (0.159 g; 1.56 mmol) and pyridine (0.198 g; 2.50 mmol). The reaction mixture was stirred at room temperature under nitrogen for 2 days. Dilute hydrochloride acid (5%) (~10 ml) was added to the solution to neutralize the remaining pyridine. Extraction of the reaction mixture was performed with ethyl acetate (6 x 10 mL), the combined organic extracts were dried over sodium sulfate and the solvent evaporated. The product was obtained as an oil (86% yield) which was homogeneous by TLC analysis.

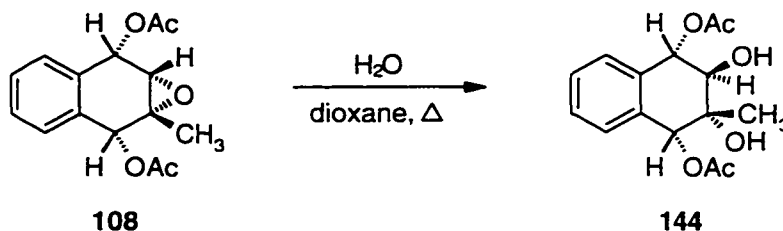
$^1\text{H NMR}$  (250MHz,  $\text{CDCl}_3$ ):  $\delta$  7.37-7.24 (m, 3H), 7.10-7.07 (m, 1H), 6.30 (s, 1H), 6.22 (s, 1H), 3.59 (d,  $J= 1.7$ , 1 H), 2.33 (s, 3H), 2.28 (s, 3H), 1.50 (s, 3H);  $^{13}\text{C NMR}$  (63MHz,  $\text{CDCl}_3$ ):  $\delta$  170.94, 170.90, 131.1, 130.0, 128.1, 128.0, 125.6, 125.4, 70.7, 68.6, 58.3, 57.4, 21.0, 20.7, 18.7

**Attempted Ring Opening of 1,4-Diacetoxy-2,3-epoxy-3-methyl-1,2,3,4-tetrahydronaphthalene 108 with Acetate**



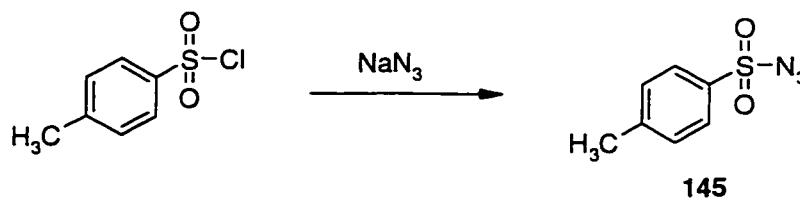
The nucleophilic ring opening of the epoxide **108** was first tried using a phase transfer catalyst (Aliquat 336) and potassium acetate. A 50 mL round bottom flask was charged with acetonitrile (5 mL), 0.1 mole equivalents of Aliquat 336 (0.011 g; 0.027 mmol) and 2 mole equivalents of potassium acetate (0.053 g; 0.54 mmol). The vessel was sealed and the mixture was stirred for 20 h. The epoxy quinone (0.075 g; 0.27 mmol) dissolved in 2 mL of acetonitrile was then added. The reaction was stirred at room temperature for 3 days. Water (30 mL) was added and extractions were performed with methylene chloride (4 x 10 mL). The organic extracts were combined, dried over sodium sulfate, and the solvent was evaporated. Analysis of the reaction mixture by  $^1\text{H}$  NMR indicated that only the starting material was present.

**Attempted Ring Opening of 1,4-Diacetoxy-2,3-epoxy-3-methyl-1,2,3,4-tetrahydronaphthalene 108 with Water**



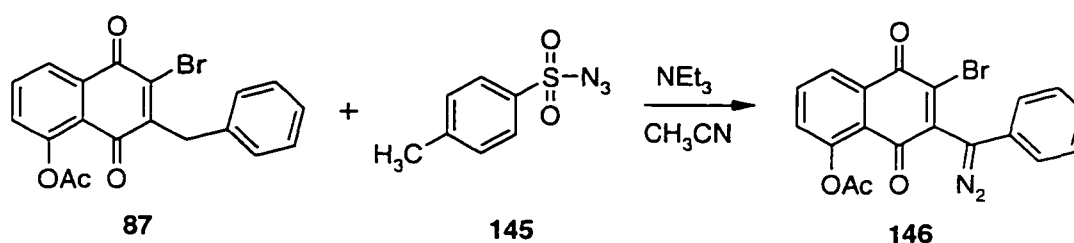
The starting material recovered as described above (0.070 g; 0.25 mmol) was dissolved in 1,4-dioxane (5 mL) and excess water (1.25 mL) was added. The solution was stirred and heated to 100°C for 18 h. The solution was then poured into water (60 mL) and extraction with ethyl acetate were performed. The combined organic extracts were dried over sodium sulfate and the solvent was evaporated. From <sup>1</sup>H NMR analysis of the crude product, it was concluded that only the starting material was present.

**Synthesis of Tosyl Azide 145**



The tosyl azide was prepared as described in the literature.<sup>80</sup> A clear colourless oil (85% yield) was obtained. This oil solidified on standing in the refrigerator. The <sup>1</sup>H NMR and IR characteristics were observed to correspond well with the literature values.<sup>80</sup>

#### Attempted Diazo Transfer to 5-Acetoxy-3-benzyl-2-bromo-1,4-naphthoquinone **87**



To a cold (0°C, ice bath) solution of 5-acetoxy-3-benzyl-2-bromo-1,4-naphthoquinone **87** (0.037 g; 0.10 mmol) in acetonitrile (1.5 mL) was added tosyl azide **145** (0.019 g; 0.10 mmol) while stirring. Triethylamine (0.041 mL; 0.3 mmol) was then added in one portion. The reaction mixture was allowed to warm to room temperature and was stirred for 12 h. Water (40 mL) was added and extraction with ethyl acetate (2 x 20 mL) was performed. The combined organic extracts were washed with an alkaline aqueous solution (0.25 g of sodium hydroxide in 10 mL of water), brine, and dried over sodium sulfate. The solvent was evaporated *in vacuo*. By observing the <sup>1</sup>H NMR of the product, it was noticed that the peak corresponding to the two non aromatic hydrogens of the benzylic group ( $\delta$  4.18) were still present. The spectrum also contained numerous other peaks suggesting that considerable decomposition had occurred.



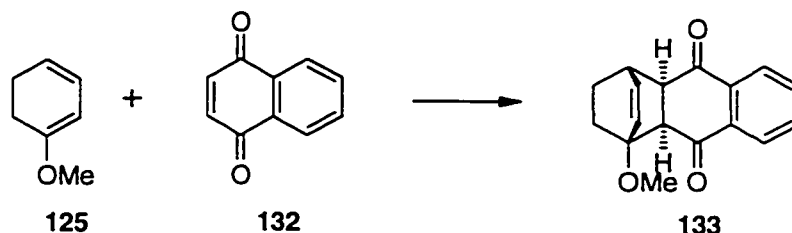
portion. The reaction mixture was allowed to warm to room temperature and was stirred for 20 h. Water (75 mL) was added and extraction with ethyl acetate (2 x 40 mL) was performed. The combined organic extracts were washed with an alkaline aqueous solution (0.5 g of sodium hydroxide in 20 mL of water), brine, and dried over sodium sulfate. The solvent was evaporated *in vacuo*.

An I.R. of the reaction mixture showed only one peak at  $2100\text{ cm}^{-1}$  in the region corresponding to the azides. This peak was far enough from the tosyl azide ( $2129\text{ cm}^{-1}$ ) to avoid confusion. The absence of tosyl azide was confirmed by TLC and  $^1\text{H}$  NMR analysis. From the  $^1\text{H}$  NMR spectrum, no peaks corresponding to the two non aromatic hydrogens of the benzylic group ( $\delta$  4.18) were obtained and no side reactions seemed to be significant although some were noted. The product obtained was in the form of an oil (0.0252 g: 40 % yield) which seemed homogeneous by TLC analysis. No attempts were made to optimize the yields.

$^1\text{H}$  NMR (250MHz,  $\text{CDCl}_3$ ):  $\delta$  8.26-8.22 (dd,  $J = 8.0\text{ Hz}, 1.2\text{ Hz}$ , 1H), 7.84-7.78 (m, 2 H), 7.47- 7.29 (m, 6 H), 2.47 (s, 3H);  $^{13}\text{C}$  NMR (63MHz,  $\text{CDCl}_3$ ):  $\delta$  179.9, 177.8, 169.5, 144.6, 136.4, 135.0, 133.8, 131.2, 129.8, 129.2, 128.3, 127.9, 125.7, 124.8, 120.5, 66.7, 21.2;  
I.R. ( $\text{cm}^{-1}$ ) 2100, 1767, 1670.

## 7.4 Synthesis Studies Towards the Cysfluoretin Model

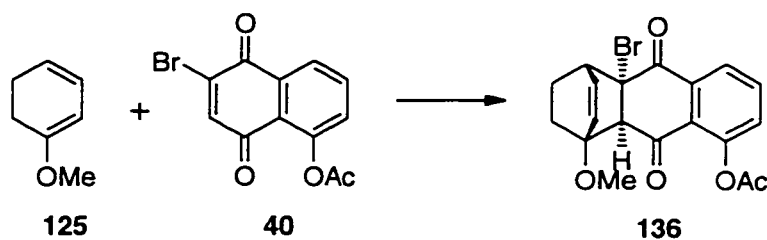
### Synthesis of the Diels-Alder Adduct Between 1,4-Naphthoquinone **132** and 1-Methoxy-1,3-cyclohexadiene **125**



To a solution of the quinone **132** (3.00 g; 19.0 mmol) dissolved in methylene chloride (120 mL) was added 3 mole equivalents of 1-methoxy-1,3-cyclohexadiene **125** (9.66 g, 57.0 mmol). The solution was stirred at room temperature for 20 h. The solvent was removed *in vacuo* and the product was recrystallized from methylene chloride/ hexane to yield a white powder (3.05 g; 60% yield).

$^1\text{H}$  NMR (250MHz,  $\text{CDCl}_3$ ):  $\delta$  7.89-7.80 (m, 2H), 7.68-7.58 (m, 2H), 5.99-5.89 (m, 2H), 3.54 (d,  $J=8.9$  Hz, 1H), 3.47 (s, 3H), 3.32 (dd,  $J=8.9, 2.8$  Hz, 1H), 3.1 (m, 1H), 2.05-1.85 (m, 2H), 1.59-1.41 (m, 2H);  $^{13}\text{C}$  NMR (63MHz,  $\text{CDCl}_3$ ):  $\delta$  197.6, 195.6, 137.4, 136.2, 135.4, 134.1, 133.5, 131.0, 126.8, 126.1, 79.7, 52.2, 51.7, 51.1, 36.6, 28.8, 24.6; I.R. ( $\text{cm}^{-1}$ ) 1680; EIMS  $m/z$  (%) 110 (100); CI- $\text{NH}_3$   $m/z$  (%) 269 (65) ( $\text{M}+1$ ) $^+$ , 268 (5), 110 (100).

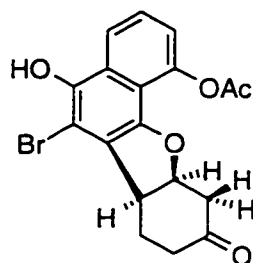
**Synthesis of the Diels-Alder Adduct Between 5-Acetoxy-2-bromo-1,4-naphthoquinone 40 and 1-Methoxy-1,3-cyclohexadiene 125**



To a solution of the quinone **40** (3.00 g; 10.2 mmol) dissolved in methylene chloride (120 mL) was added 3 mole equivalents of 1-methoxy-1,3-cyclohexadiene (5.19 g, 30.6 mmol). The solution was stirred at room temperature for 20 h. The solvent was removed *in vacuo* and the product was recrystallized from methylene chloride/ hexane to yield an off white powder ( 2.18 g; 53% yield).

$^1\text{H NMR}$  (250MHz,  $\text{CDCl}_3$ ):  $\delta$  7.84 (d,  $J= 8.0$  Hz, 1H), 7.70 (t,  $J= 7.6$  Hz, 1H), 7.31 (d,  $J=8.0$  Hz, 1H), 6.37 (d,  $J= 8.3$  Hz, 1H), 6.18 (t,  $J=7.8$  Hz, 1H), 3.60 (s, 1H), 3.54 (s, 3H), 3.15 (m, 1H), 2.34-2.22 (m, 1H), 2.05-1.96 (m, 1H), 1.85-1.75 (m, 1H), 1.56-1.50 (m, 1H); I.R. ( $\text{cm}^{-1}$ ) 1760, 1701, 1596, 1203.

## Isolation of Cyclic Byproduct 138

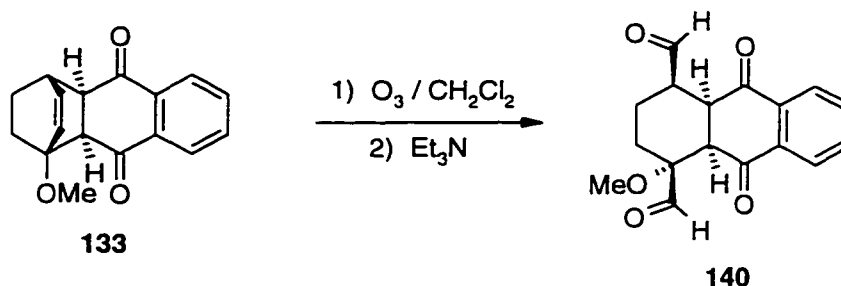


**138**

The byproduct **138** was isolated from the crude reaction mixture obtained above, in a previous experiment, when a column chromatography (20% ethyl acetate/ hexane) was used to purify the Diels-Alder adduct **136**. The byproduct **138** eluted after the Diels-Alder adduct **136**. The byproduct appeared as a brown oil (0.07g ; 5 %).

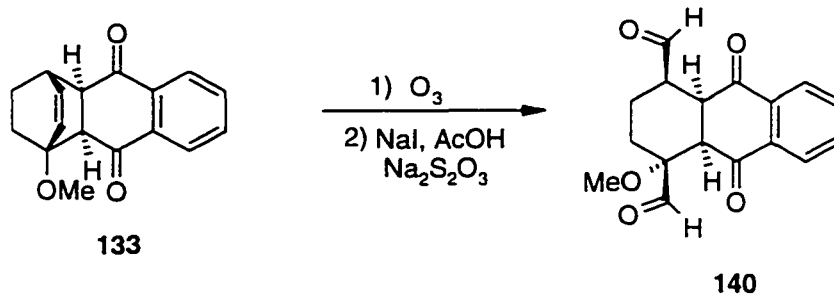
$^1\text{H NMR}$  (250MHz,  $\text{CDCl}_3$ ):  $\delta$  9.53 (s, 1H), 8.03 (d,  $J=8$  Hz, 1H), 7.45 (t,  $J= 8$  Hz, 1H), 7.13 (d,  $J= 8$  Hz, 1H), 5.35-5.28 (m, 1H), 3.81-3.74 (m, 1H), 3.04 (dd,  $J= 3$  Hz, 17 Hz, 1H), 2.75 (dd,  $J= 3$  Hz, 17 Hz, 1H), 2.19 (s, 3H), 2.02-1.92 (m, 2H), 1.84-1.63 (m, 2H)

**Attempted Ozonolysis of the Diels-Alder Adduct Between 1,4-Naphthoquinone 133 and 1-Methoxy-1,3-cyclohexadiene Using Triethylamine**



The Diels-Alder product **133** (0.130 g; 0.49 mmol) was dissolved in methylene chloride (30 mL) and the solution was chilled to  $-40^{\circ}\text{C}$  (acetone +  $\text{CO}_2$  bath). The ozone generator was set at an oxygen pressure of 11 psi, and an ozone output of 50%. After 15 min., a TLC seemed to indicate that all of the starting material was gone. The ozone generator was turned off and the flask was flushed with nitrogen for 5 min to remove all the ozone from the flask. The disappearance of all the ozone was tested with a starch iodine stick. The temperature of the bath was then decreased to  $-78^{\circ}\text{C}$  and 2 mole equivalents of triethylamine (0.10 g; 0.98 mmol) were added. The solution changed color for the first time from colorless to purple. After 5 min., the solution became orange. The bath was removed and the solution was left to warm to room temperature for 1 hour. The solvent was removed *in vacuo*. TLC analysis indicated that the product was a complex mixture. A silica gel column (20% ethyl acetate/hexane) was used to try to purify the reaction mixture but no compound of interest was isolated. NMR of the fractions obtained indicated that each was still a complex mixture.

**Attempted Ozonolysis of Diels-Alder Adduct Between 1,4-Naphthoquinone 133 and 1-Methoxy-1,3-cyclohexadiene Using NaI/ AcOH, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>**



The Diels-Alder product **133** (0.060 g; 0.23 mmol) was dissolved in methylene chloride (25 mL) and the solution was chilled to  $-40^{\circ}\text{C}$  (acetone + CO<sub>2</sub> bath). The ozone generator was set with an oxygen flow of 20 scfh, an oxygen pressure of 11 psi, and an ozone output of 50%. After 35 min., a TLC seemed to indicate that all of the starting material was gone. The ozone generator was turned off and the flask was flushed with nitrogen for 5 min to remove all the ozone from the flask. The disappearance of all the ozone was tested with a starch iodine stick. The temperature of the bath was then decreased to  $-78^{\circ}\text{C}$  and 4 mole equivalent of sodium iodide (0.135 g; 0.906 mmol) dissolved in acetic acid (10 mL) was added. The colorless solution changed color gradually to yellow, wine red, and ended up dark brown. After 30 min, a saturated aqueous solution of sodium thiosulfate (35 mL) was added. The solution turned suddenly yellow-white and opaque. The bath was removed and the solution was left to warm to room temperature. The reaction mixture was diluted with water (40 mL) and extractions were performed with methylene chloride (3 x 40 mL). The yellow organic extracts were combined, washed with an aqueous saturated NaHCO<sub>3</sub> solution

(50 ml) and brine (60 mL). The organic phase was then dried over sodium sulfate, and the solvent was removed *in vacuo*. A  $^1\text{H}$  NMR of the crude product was recorded and suggested that extensive decomposition had occurred. The TLC obtained of the crude mixture also suggested that a complex mixture had formed. A very small peak was observed at 14 ppm in the  $^1\text{H}$  NMR spectrum. A silica gel column (40% ethyl acetate/hexane) was used to try to separate the mixture. No compound of interest was isolated.  $^1\text{H}$  NMR analysis indicated that each was still a complex mixture.

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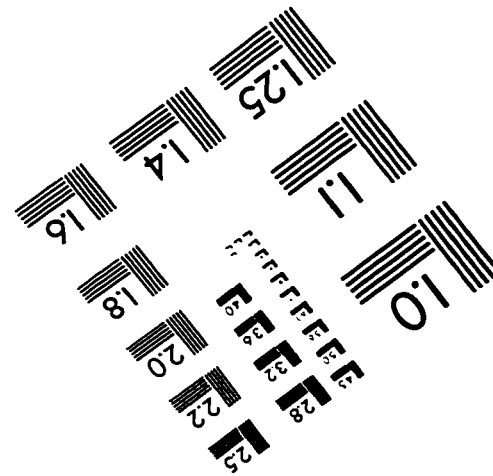
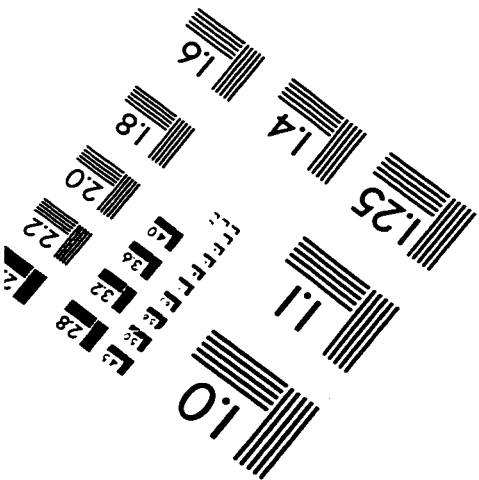
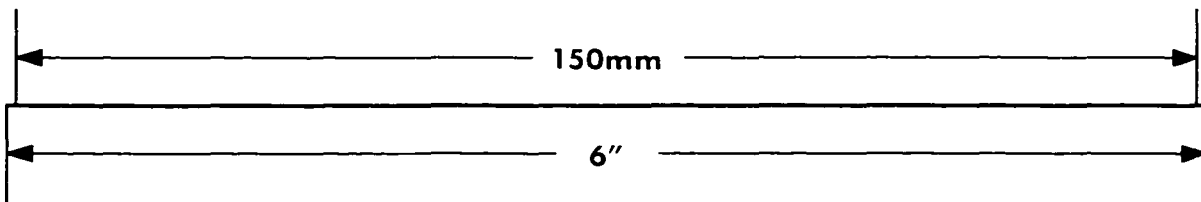
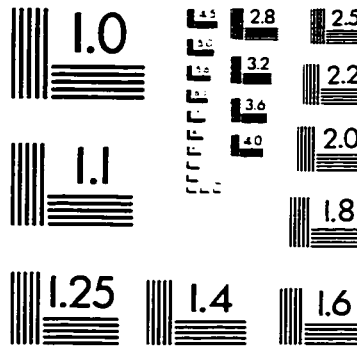
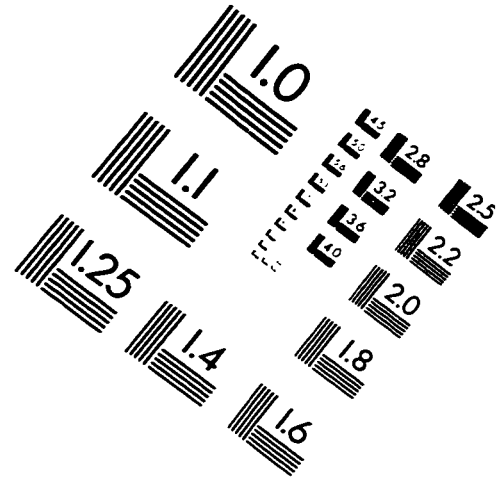
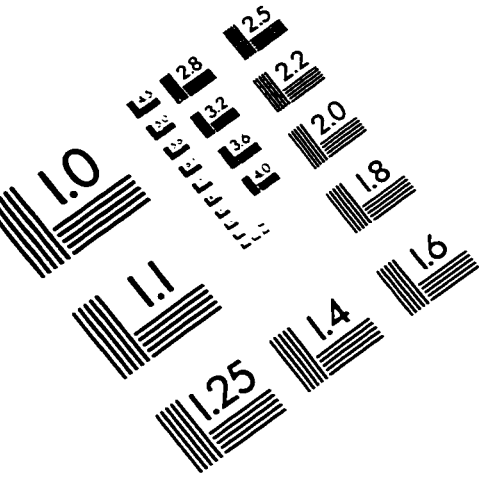
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