Synthesis of an α,3-Dehydrotoluene Biradical Precursor with DNA Cleaving Activity and Studies Directed Toward the Total Synthesis of Tetracycline

Thesis by
Cynthia Ann Parrish

In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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(Submitted October 19, 1998)
In memory of my loving grandparents...

Somehow, I know you are cheering for me right now.
Acknowledgments

I would like to thank my advisor, Andy Myers, for his guidance and support throughout my graduate career. Although he provided me with substantial advice for my projects, he challenged me further in allowing me a large degree of independence in my research endeavors. I am grateful for the opportunity to work in his laboratories on two very different and exciting projects. And, from 3,000 miles away, I am especially thankful for his continued support during the time it took to write this thesis.

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Abstract

The synthesis and characterization of a model of the enediyne antibiotics is described. The prepared conjugate consists of an α,3-dehydrotoluene biradical precursor tethered to an N-methylpyrrolecarboxamide minor groove binding element. The conjugate is shown to bind and cleave DNA with sequence selectivity. The binding domain is shown to localize the allene-ene-yne effector domain for sequence-selective DNA cleavage at micromolar concentrations of substrate. The time course of DNA cleavage parallels the rate of cyclization of the bioconjugate in organic solvent to form an α,3-dehydrotoluene biradical. These results indicate that the (Z)-allene-ene-yne functional group is a viable effector domain for the cleavage of DNA upon mild thermal activation.

Synthetic studies directed toward a concise and versatile synthesis of the antibiotic tetracycline are described. A strategy based on an isobenzofuran Diels–Alder cycloaddition to assemble the two halves of tetracycline is presented. The synthesis of the phthalide left-hand half is shown in five steps with 56% overall yield from commercially available starting materials. Several isobenzofuran Diels–Alder reactions are described that model the proposed condensation of the two halves of tetracycline. Specifically, a thermal Diels–Alder reaction is successfully demonstrated with an enone dienophile containing an α-ester functional group. The synthesis of 6-dimethylaminomethyl-2,2-dimethyl-1,3-dioxin-4-one as a protected and fully functionalized right-hand half of the A ring of tetracycline is described. Strategies are discussed that aim to utilize this substrate in the synthesis of the right-hand half of tetracycline. A novel and potentially rapid route involving intermediate 2,4- or 2,5-cyclohexadienones from the oxidation of phenol precursors is briefly examined.
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<td>A</td>
<td>adenine</td>
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<tr>
<td>Å</td>
<td>angstrom</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2'-azobis(isobutyronitrile)</td>
</tr>
<tr>
<td>APT</td>
<td>attached proton test</td>
</tr>
<tr>
<td>bp</td>
<td>base pair(s)</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>C</td>
<td>cytosine</td>
</tr>
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<td>°C</td>
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<td>cat.</td>
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<tr>
<td>CDI</td>
<td>1,1'-carbonyldiimidazole</td>
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<td>CHD</td>
<td>1,4-cyclohexadiene</td>
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<tr>
<td>CI</td>
<td>chemical ionization</td>
</tr>
<tr>
<td>cm⁻¹</td>
<td>reciprocal centimeters</td>
</tr>
<tr>
<td>cpm</td>
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</tr>
<tr>
<td>δ</td>
<td>chemical shift (parts per million)</td>
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<td>DABCO</td>
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<tr>
<td>dec</td>
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<td>DIPEA</td>
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<tr>
<td>DMF</td>
<td><em>N</em>,<em>N</em>-dimethylformamide</td>
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<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<td>DNA</td>
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<td>EWG</td>
<td>electron-withdrawing group</td>
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<td>FAB</td>
<td>fast atom bombardment</td>
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<td>FT</td>
<td>Fourier transform</td>
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<td>g</td>
<td>gram</td>
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<tr>
<td>G</td>
<td>guanine</td>
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<td>J</td>
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<td>KOt-Bu</td>
<td>potassium tert-butoxide</td>
</tr>
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<td>LDA</td>
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<td>M</td>
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<td>m-CPBA</td>
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</tr>
<tr>
<td>mg</td>
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</tr>
<tr>
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<tr>
<td>mp</td>
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<td>MPE</td>
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<td>MTAD</td>
<td>4-methyl-1,2,4-triazoline-3,5-dione</td>
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<td>µL</td>
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<tr>
<td>n</td>
<td>normal</td>
</tr>
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<td>N</td>
<td>normal (concentration)</td>
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<tr>
<td>nm</td>
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<tr>
<td>NMR</td>
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</tr>
<tr>
<td>nOe</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>o</td>
<td>ortho</td>
</tr>
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<td>o.d.</td>
<td>outer diameter</td>
</tr>
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<td>pH</td>
<td>hydrogen ion concentration (log scale)</td>
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<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>Pr</td>
<td>propyl</td>
</tr>
<tr>
<td>psi</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>R</td>
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</tr>
<tr>
<td>$R_f$</td>
<td>retention factor</td>
</tr>
<tr>
<td>rp</td>
<td>reverse-phase</td>
</tr>
<tr>
<td>s</td>
<td>seconds</td>
</tr>
<tr>
<td>S</td>
<td>sinister</td>
</tr>
<tr>
<td>SEM</td>
<td>2-(trimethylsilyl)ethoxymethyl</td>
</tr>
<tr>
<td>t</td>
<td>tertiary</td>
</tr>
<tr>
<td>T</td>
<td>thymine</td>
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<tr>
<td>$t_{1/2}$</td>
<td>half-life</td>
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<tr>
<td>TBAF</td>
<td>tetrabutylammonium fluoride</td>
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<tr>
<td>TBHP</td>
<td>tert-butyl hydroperoxide</td>
</tr>
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</tr>
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<td>TDS</td>
<td>tert-butylphenylsilyl</td>
</tr>
<tr>
<td>Tf</td>
<td>trifluoromethanesulfonate</td>
</tr>
<tr>
<td>TFA</td>
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<td>TFAA</td>
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</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<tr>
<td>TIPS</td>
<td>triisopropylsilyl</td>
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<td>TLC</td>
<td>thin layer chromatography</td>
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<td>TMS</td>
<td>trimethylsilyl</td>
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<td>Definition</td>
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<td>---------------------------------</td>
</tr>
<tr>
<td>Tris</td>
<td>tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>$p$-Ts</td>
<td>para-toluenesulfonyl</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>v/v</td>
<td>volume-to-volume ratio</td>
</tr>
<tr>
<td>w/w</td>
<td>weight-to-weight ratio</td>
</tr>
<tr>
<td>Z</td>
<td>zusammen</td>
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Chapter 1

Synthesis of an α,3-Dehydrotoluene Biradical Precursor with DNA Cleaving Activity
Introduction

The naturally occurring enediyne antitumor antibiotics are a structurally unique class of molecules possessing potent DNA cleaving ability. Their biological activity arises from the chemical activation of a highly unsaturated precursor, which subsequently undergoes cyclization to generate a reactive biradical. This intermediate is capable of abstracting hydrogen atoms from the deoxyribose backbone of double-stranded DNA in a site-specific manner. To further characterize and understand the mode of action of the enediyne antibiotics, much effort has been directed towards the construction of model compounds capable of biradical formation and thus having the potential for DNA cleavage.

Several novel compounds which produce 1,4-biradical intermediates upon both chemical and thermal activation have been reported from this group. In an effort to establish and characterize any DNA cleaving capabilities these systems may possess, a model substrate was envisioned consisting of three distinct portions: (1) a latent biradical precursor; (2) an agent with recognized affinity for DNA to provide the biradical precursor with similar DNA binding properties; and (3) a tether to connect these two moieties. Herein is described a completely synthetic model of the enediyne antibiotics which, upon thermal activation, gives rise to an α,3-dehydrotoluene biradical capable of site-selective DNA cleavage.

Background

The (Z)-allene-ene-yne functional group has been shown to undergo a facile unimolecular thermal isomerization reaction to form an α,3-dehydroalkylbenzene biradical intermediate. The cycloaromatization of (Z)-1,2,4-heptatriene-6-yne (1) to α,3-dehydrotoluene (2) may be considered prototypical, occurring with a half-life of about one day at 37 °C. The α,3-dehydroalkylbenzene biradicals produced in these cyclization reactions exhibit reactivity that is consistent with traditional definitions of both radical and polar (zwitterionic) species. For example, the thermal cyclization of (Z)-allene-ene-yne 3
in methanol leads to the formation of products consistent with a free radical description of the biradical intermediate, the bibenzyl derivative 4 (1% yield) and the alcohol 5 (7% yield), in addition to the methyl ether 6 (39% yield), a product anticipated from a polar description of the biradical intermediate wherein the benzylic carbon is $\delta^+$ and the $m$-aryl carbon is $\delta^-$ (Scheme I). The distribution of polar versus free radical products varies with subtle changes in the reaction medium in an entirely predictable fashion. Substitution of deuterium for protium on the methanol carbon (CD$_3$OH) leads to the exclusive formation of the product derived from a polar reaction mechanism, the (deuterated) methyl ether 6-$d_3$, while substitution of the hydroxylic proton of methanol with deuterium (CH$_3$OD) skews the product distribution towards that of a free radical reaction mechanism (Scheme I).

The ability of $\alpha,3$-dehydroalkylbenzene biradicals to abstract a hydrogen atom from the methyl group of methanol raises the possibility that these species might function as DNA damaging agents, in analogy to the biradical intermediates arising from the enediyne antibiotics. However, the variation of the branching ratio between radical and polar reaction pathways with relatively small changes in the bond dissociation energy of the hydrogen atom donor and with the acidity of the medium makes it difficult to predict just how an $\alpha,3$-dehydrotoluene intermediate might react with double-stranded DNA in an aqueous environment. It is easy to imagine that the primary reaction pathway might involve the addition of water by a polar mechanism. On the other hand, if the biradical were formed in the minor groove of DNA, then it may well react in a manner analogous to the enediyne antibiotics and abstract a hydrogen atom from the ribose backbone of DNA. In a preliminary effort to explore this issue, the (Z)-allene-ene-yne 3 was heated at 60 °C in 20% aqueous tetrahydrofuran. The purpose of the experiment was to see if the biradical
Scheme I

\[
\begin{align*}
\text{TDSO} & \text{CH}_2 \text{OCH}_3 \\
\text{TDSO} & \text{CH}_2 \text{CD} \text{OCD}_3 \\
\text{TDSO} & \text{CH}_2 \text{OD} \\
\end{align*}
\]

\[
\begin{align*}
\text{TDS} = \text{tert-butyldiphenylsilyl}
\end{align*}
\]
intermediate would react preferentially with water in a polar mechanism or by hydrogen atom abstraction from the labile \( \alpha \)-CH bonds of tetrahydrofuran. In the event, only products arising from a free radical reaction mechanism (adducts 4, 7, and 8) were observed. Encouraged by this result, the goal became to address in a more direct fashion the question of whether an \( \alpha,3 \)-dehydrotoluene intermediate might lead to free radical damage of double-stranded DNA.

Because \( \alpha,3 \)-dehydrotoluene itself was anticipated to have a low affinity for double-stranded DNA (a speculation verified below), the well-established \( N \)-methylpyrrolecarboxamide DNA binding motif of the natural products distamycin and netropsin was incorporated covalently into the substrates of this work. The motif binds tightly in the minor groove of double-stranded DNA and displays high specificity for AT-rich regions of DNA. This binding motif was first utilized by Dervan and co-workers in their development of the affinity cleavage concept and in the design of DNA-cleaving molecules. It has subsequently been used for the synthesis of several bioconjugates incorporating biradical precursors (Figure 1). With these substrates, it has been demonstrated that addition of the netropsin binding unit enhances cleavage ability versus control studies conducted with the model enediyne lacking a minor groove binding element. Typically, such bioconjugates have been shown to exhibit DNA cleavage in a DNA nicking
Figure 1. Examples of enediyne model systems linked to $N$-methylpyrrolecarboxamide binding motifs.$^{9-11}$
assay, and thus the particulars of DNA binding and the site-selectivity of cleavage are generally not probed. The synthesis of conjugate 9, consisting of an α,3-dehydrobenzene precursor covalently linked to an N-methylpyrrolecarboxamide minor groove binding element, and its DNA-binding and -cleaving properties are described herein.

**Synthesis and Characterization of Conjugate 9**

The (Z)-allene-ene-yne alcohol 15 was prepared using methodology previously developed for the synthesis of 3 (Scheme II). Protection of the known alcohol 10 with tert-butyldimethylsilyl chloride (1.1 equiv), triethylamine (1.5 equiv), and 4-dimethylaminopyridine (0.27 equiv) in dichloromethane afforded the silyl ether 11 in 94% yield. Palladium-catalyzed coupling (cuprous iodide (0.15 equiv), bis(triphenylphosphine)palladium(II) chloride (0.05 equiv), n-propylamine (4.0 equiv), THF) of 11 with propargyl alcohol (2.0 equiv) produced the (Z)-enediyne 12 (80%). Protodesilylation of the acetylenic trimethylsilyl group of 12 was accomplished in 89% yield upon addition of a sodium hydroxide solution (50% w/w) to 12 in ice-chilled methanol. The resulting alcohol 13 was transformed into the allene 14 using methodology previously developed for this purpose. Thus, activation of the hydroxyl group of 13
with methanesulfonyl chloride (3.0 equiv) and triethylamine (5.0 equiv) in dichloromethane (0 °C) and displacement of the resulting mesylate with hydrazine in methanol (31.8 equiv) afforded a propargylic hydrazine intermediate. Extractive isolation of this intermediate and oxidation of the crude extract with 4-methyl-1,2,4-triazoline-3,5-dione (2.0 equiv) in diethyl ether (−78 °C) produced the allene 14 in 40% yield from alcohol 13. Cleavage of the tert-butyltrimethylsilyl ether group of 14 (triethylamine trihydrofluoride, CH₂Cl₂) then provided the biradical precursor 15 in 76% yield.

The nitrodistamycin derivative 16 is typically linked to an effector molecule via its hydrogenation product, the air-sensitive amine 17. Dervan and co-workers, as well as others, have shown that the proper choice of a linking group can be a critical component in the design of DNA-cleaving agents. Initially, a succinyl linker was chosen to couple the
binding and effector domains, but it was found that the resulting conjugate 18 was unstable, readily cyclizing to form the imide 19 by expulsion of the alcohol 15.

This problem was avoided by using a fumarate linking group. Activation of fumaric acid as its bis(pentafluorophenyl) ester 20 was accomplished in 63% yield by bis-coupling with pentafluorophenol (3.0 equiv) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.0 equiv) and DMAP (0.04 equiv) in dichloromethane (Scheme III). Addition of diester 20 (3.0 equiv) to a solution of alcohol 15 in dichloromethane containing triethylamine (5.0 equiv) and DMAP (0.01 equiv) gave rise to
the activated ester 21 (60%). When this product (1.0 equiv) was stirred with a solution of amine 17 (freshly prepared by hydrogenation of 16 with 10% palladium on carbon in methanol) in N,N-dimethylformamide, the conjugate 9 was formed in 36% yield (from 16) after purification on silica gel using 1:1 acetonitrile–methanol containing 1% concentrated ammonium hydroxide solution as the eluent.

Scheme III

Thermal cyclizations of conjugate 9 (3.0–3.8 mM) were conducted at 37 °C in dimethyl sulfoxide-d₆ as the solvent containing 1,4-cyclohexadiene (30–300 mM) and N,N-dimethylformamide (25 mM, internal standard) in NMR tubes that had been sealed in vacuo. In a typical experiment, run to ~85% completion, the cyclization products 22 (25%) and 23 and 24 (inseparable mixture, 25% combined yield) were isolated by high-performance liquid chromatography (HPLC). A similar distribution of products had been observed in an earlier study of the cyclization of 3. The rate of cyclization of 9 at 37 °C was determined by ¹H NMR monitoring of the reaction as a function of time. Analysis of the data (Figure 2) established that 9 had decayed in a first-order manner with a rate constant $k = (1.75 ± 0.46) \times 10^{-5}$ s⁻¹ ($t_{1/2} = 10.9$ h, 37 °C). This rate is somewhat faster than the rate of cyclization of 3 under similar conditions ($8.94 \times 10^{-6}$ s⁻¹, $t_{1/2} = 21.5$ h, 37
Figure 2. Plot of kinetic data for cyclization of 9 at 37 °C in dimethyl sulfoxide-$d_6$ containing 1,4-cyclohexadiene.
°C);\textsuperscript{21} however, with experimental uncertainty, it is unclear if there is, in fact, a meaningful variance in the two rates.

DNA Binding and Cleavage Studies

The binding of conjugate 9 to double-stranded, B-form DNA was evaluated using the MPE•Fe(II) footprinting reagent of Dervan and co-workers\textsuperscript{7} by determining the ability of 9 to protect against DNA cleavage within a 517 bp restriction fragment of plasmid pBR322 labeled, in separate experiments, with $^{32}$P at the 5' and 3' termini. Dervan and co-workers have shown that this restriction fragment contains two preferred sites for the binding of the distamycin analog 25.\textsuperscript{22} Footprinting reactions were performed in
association buffer (10 mM NaCl, 25 mM Tris-acetate, pH 7.0) in the presence of double-stranded calf thymus DNA (100 µM bp) at 37 °C across a range of concentrations of 9 (50–0.5 µM) and were evaluated by gel electrophoresis using standard phosphor-imaging technology to reveal "footprints" of protection versus an MPE•Fe(II) control lane (Figure 3). Plotting of the data in histogram format shows that conjugate 9, at a concentration of 10 µM, protects two 5 bp sequences (5'-TTTTT-3' and 5'-AATAA-3') from cleavage by MPE•Fe(II) (Figure 4A). These sequences correspond to the areas of protection observed for the distamycin analog 25 and establish that the binding domain serves to confer sequence specific DNA binding to conjugate 9, as anticipated.

Quantitative MPE•Fe(II) footprinting titrations were performed to compare the relative DNA-binding affinities of the conjugate 9 and the distamycin analog 25 at the two designated protection sites. Footprinting reactions (as above) were conducted over a wide range of substrate concentrations (500 µM to 1 nM for 9; 100 µM to 0.1 nM for 25) and were analyzed by gel electrophoresis using standard phosphor-imaging technology to evaluate the data. From these data, binding affinities were calculated at each of the two binding sites (5'-TTTTT-3' and 5'-AATAA-3') for each substrate (the binding affinities are reported as relative, and not absolute, values due to the presence of calf thymus DNA in
Figure 3. Autoradiogram of an 8% denaturing polyacrylamide gel showing MPE•Fe(II) footprinting of 9. All reaction mixtures contain calf thymus DNA (100 µM bp), the end-labeled 517 bp restriction fragment of plasmid pBR322 (~18,000 cpm), and NaCl (10 mM) in Tris-acetate (25 mM, pH 7.0) buffer. Lanes 1–9 (3'-labeled DNA) contain intact DNA, A reaction,23 Maxam–Gilbert G reaction,24 and 0, 50, 20, 10, 2, and 0.5 µM substrate 9, respectively. Lanes 10–18 (5'-labeled DNA) contain intact DNA, A reaction,23 Maxam–Gilbert G reaction,24 and 0, 50, 20, 10, 2, and 0.5 µM substrate 9, respectively. Lanes 4–9 and 13–18 also contain MPE•Fe(II) (4 µM) and dithiothreitol (4 mM).
Figure 4. (A) Histogram of footprinting patterns of protection by 9 (10 µM, 37 °C, 15–25 min) from MPE•Fe(II) cleavage within a 517 bp restriction fragment of pBR322. Boxed sequences represent binding sites. 25 Shaded bars are proportional to the extent of protection from MPE•Fe(II) cleavage at the indicated base pair site. (B) Patterns of cleavage arising from 9 (10 µM, 37 °C, 60–65 h) within the same 517 bp restriction fragment of pBR322. Boxed sequences designate the binding sites defined in the legend for (A) above. Arrow heights are proportional to the extent of cleavage at the indicated base pair site.
these experiments, Table 1). Inspection of the data shows that conjugate 9 binds with higher affinity at the 5'-TTTTT-3' site than at the 5'-AATAA-3' site, the same order of binding preference displayed by distamycin analog 25. Importantly, the data of Table 1 show that 9 binds some 30 times more weakly than the distamycin analog 25 (5'-AATAA-3' site), despite the larger size and greater hydrophobicity of 9. This may reflect a poor fit of the allene-ene-yne cleaving domain of 9 into the minor groove of DNA, a feature which might explain, in part, the poor DNA cleavage efficiency of 9 (vide infra). Variation of the linking domain may well lead to improved DNA binding (and cleavage efficiency) in future conjugate molecules.

**Table 1.** Relative binding affinities of 9 and 25.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>5'-TTTTT-3'</th>
<th>5'-AATAA-3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>distamycin analog 25</td>
<td>212</td>
<td>29</td>
</tr>
<tr>
<td>conjugate 9</td>
<td>2.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Determined from binding isotherms generated from three separate MPE•Fe(II) quantitative footprinting experiments comparing the binding of 9 and 25 under identical conditions (100 µM calf thymus DNA, 25 mM Tris-acetate (pH 7.0), 10 mM NaCl).

The ability of 9 to cleave double-stranded B-form DNA was probed using the 3'- and 5'-32P-labeled 517 bp restriction fragment of plasmid pBR322 described above in the footprinting studies. Cleavage reactions employed the conjugate 9 (50–0.5 µM), calf thymus DNA (100 µM bp), and 3'- or 5'-radiolabeled restriction fragment (~18,000 cpm) and were performed in association buffer (10 mM NaCl, 25 mM Tris-acetate, pH 7.0) at 37 °C for 60–65 h. DNA cleavage was assayed by gel electrophoresis using standard phosphor-imaging technology to quantitate cleavage at each base pair site (Figures 5 and 6). The gel data are translated into histogram format in Figure 4B. The minimum cleavage
Figure 5. DNA cleavage brought about by thermal reaction (37 °C, 60–65 h) with bioconjugate 9. Autoradiogram of an 8% denaturing polyacrylamide gel. All reaction mixtures contain calf thymus DNA (100 µM bp), the 3'-labeled 517 bp restriction fragment (~18,000 cpm), and NaCl (10 mM) in Tris-acetate (25 mM, pH 7.0) buffer. Lanes 1–8 contain intact DNA, A reaction, 23 Maxam–Gilbert G reaction, 24 and 50, 20, 10, 2, and 0.5 µM substrate 9, respectively.
Figure 6. DNA cleavage brought about by thermal reaction (37 °C, 60–65 h) with bioconjugate 9. Autoradiogram of an 8% denaturing polyacrylamide gel. All reaction mixtures contain calf thymus DNA (100 µM bp), the 5'-labeled 517 bp restriction fragment (~18,000 cpm), and NaCl (10 mM) in Tris-acetate (25 mM, pH 7.0) buffer. Lanes 1–8 contain intact DNA, A reaction,23 Maxam–Gilbert G reaction,24 and 50, 20, 10, 2, and 0.5 µM substrate 9, respectively.
efficiency, calculated by integration of all migrating radiolabeled fragments against total radioactivity, was 0.2%. This value contrasts with cleavage efficiencies of 5.7% for dynemicin A, 28 25% for neocarzinostatin chromophore, 29 and ~90% for calicheamicin γ1, 30 all members of the enediyne class of natural products, and, as discussed above, may be due, in part, to a poor fit of the effector domain into the minor groove of DNA. The observed patterns of cleavage by 9 do not resemble typical patterns produced by diffusible cleaving agents and are site selective, exhibiting a preference for the 3'-side of each of the two binding sites defined in the footprinting studies above. This contrasts with the behavior of the affinity cleaving agent distamycin•EDTA, 31 which produces a "Gaussian" cleavage pattern typical of diffusible cleaving agents and shows a bias for cleavage toward the 5' side of the 5’-AATAA-3' site. The time course of DNA cleavage by 9 was studied by gel electrophoresis of a parallel series of cleavage reactions conducted as above, but with varied reaction periods (0–60 h). Analysis of the data shows that DNA cleavage increases linearly for the first 20 h of the reaction before slowing. This time course parallels the observed half-life for the cyclization of 9, as required by a mechanism wherein DNA cleavage is brought about by an α,3-dehydrotoluene intermediate.

The sequence selectivity of DNA cleavage by 9 exhibits an interesting variation as a function of the concentration of 9 (Figures 5 and 6). At the highest concentration of 9 (50 µM), the cleavage pattern is clearly shifted versus that produced by a reaction employing 10 µM 9. One explanation for this shift could be that more than one conjugate molecule binds to the DNA-binding site at higher concentrations of conjugate. The binding of distamycin analogs as dimeric species at elevated concentrations has precedent. 32 Shifting of the protection pattern in footprinting studies of 9 (Figure 3) is also evident at the highest concentration of 9 (50 µM).

The evaluation of single-stranded versus double-stranded DNA cleavage products from the reaction of conjugate 9 with the 3'-radiolabeled 517 bp restriction fragment, as described above, was conducted by non-denaturing gel electrophoresis. There was no
evidence of double-stranded DNA cleavage (data not shown). This result is consistent with the idea that DNA cleavage is brought about by an α,3-dehydrotoluene biradical with a single highly reactive radical site; however, given the poor cleavage efficiency overall, the likelihood of a double-stranded nick is remote from a simple probabilistic view as well.

As a control, the parent (Z)-allene-ene-yne alcohol 15 was tested for DNA cleaving activity. Cleavage trials were performed over a range of concentrations of 15 (100–10 mM) with the 3'-radiolabeled 517 bp DNA restriction fragment of pBR322 described above and calf thymus DNA (100 µM bp) in association buffer (10 mM NaCl, 25 mM Tris-acetate, pH 7.0) at 37 °C for 60–65 h and were evaluated using gel electrophoresis (Figure 7). DNA cleavage with 15 was apparent only at concentrations of 25 mM or greater, or some 2000 times higher than concentrations necessary for observable cleavage by the conjugate 9. In addition, the cleavage pattern exhibited a preference for cleavage at A and G sites, suggesting a possible change in the cleavage mechanism, e.g., alkylative depurination. The data strongly support the view that the DNA-binding element is critical for the sequence-specific cleavage of DNA by 9 at micromolar concentrations.

Conclusions

The studies presented here describe the synthesis and characterization of bioconjugate 9 as a mimic of the enediyne antibiotics. The conjugate 9 has been shown to bind and cleave DNA with sequence selectivity. The binding domain has been shown to localize the allene-ene-yne effector domain for sequence-selective DNA cleavage at micromolar concentrations of substrate. The time course of DNA cleavage parallels the rate of cyclization of 9 to form an α,3-dehydrotoluene biradical. The pattern of DNA cleavage by 9 is suggestive of a nondiffusible cleaving agent. Although the data are consistent with the view that DNA cleavage arises from hydrogen-atom abstraction by a localized α,3-dehydrotoluene biradical, the mechanism of DNA cleavage by 9 has by no means been established. Importantly, it has been shown that the (Z)-allene-ene-yne functional group is
Figure 7. DNA cleavage brought about by thermal reaction (37 °C, 60–65 h) with alcohol 15. Autoradiogram of an 8% denaturing polyacrylamide gel. All reaction mixtures contain calf thymus DNA (100 μM bp), the 3'-labeled 517 bp restriction fragment (~18,000 cpm), and NaCl (10 mM) in Tris-acetate (25 mM, pH 7.0) buffer. Lanes 1–8 contain intact DNA, A reaction, Maxam-Gilbert G reaction, and 100, 75, 50, 25, and 10 mM substrate 15, respectively.
a viable effector domain for DNA cleavage upon mild thermal activation. The efficiency of DNA cleavage by 9 is poor and may reflect less than optimum positioning of the effector domain in the minor groove of DNA. This speculation receives some support from the fact that the conjugate 9 binds ca. 30 times more weakly to DNA than the smaller, less hydrophobic distamycin analog 25. This observation also suggests that future studies may benefit from modification of the linking domain.
Experimental Section

General Procedures. All reactions were performed in flame-dried round bottom or modified Schlenk (Kjeldahl shape) flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Where necessary (so noted), reaction mixtures were degassed by alternate evacuation/argon-flush cycles (five or more iterations). Organic solutions were concentrated by rotary evaporation at ~25 Torr (water aspirator). Flash chromatography was performed as described by Still et al.,\textsuperscript{33} employing 230–400 mesh silica gel. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with 0.25 mm 230–400 mesh silica gel containing a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light and/or exposure to an acidic solution of p-anisaldehyde followed by heating on a hot plate. NMR tubes for sealed tube experiments were fabricated by attachment of a standard 18 cm [5 mm outside diameter (o.d.)] NMR tube to a standard taper female 14/20 joint using a 7 cm extension of 5 mm o.d. medium-wall borosilicate glass. Sealed NMR samples were prepared by flame-sealing a frozen (liquid nitrogen), evacuated ($\leq$10 mTorr) sample that had been degassed by five successive freeze–pump–thaw cycles.

Materials. Commercially available reagents were used as received with the following exceptions. Diethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl. Benzene, toluene, dichloromethane, triethylamine, $N,N$-diisopropylethylamine, and hexanes were distilled from calcium hydride at 760 Torr. Methanol was distilled from magnesium at atmospheric pressure. Methanesulfonyl chloride was distilled from phosphorus pentoxide at atmospheric pressure. Anhydrous $N,N$-dimethylformamide was purchased from Aldrich Chemical Co. and was stored over 4Å molecular sieves. Cuprous iodide was purified by continuous extraction (48 hours)
with tetrahydrofuran in a Soxhlet apparatus. 1,4-Cyclohexadiene was purified by passage through a short column of neutral alumina immediately prior to use.

**Instrumentation.** Infrared (IR) spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrophotometer internally referenced to a polystyrene standard. Data are presented as follows: frequency of absorption (cm$^{-1}$), intensity of absorption ($s =$ strong, $m =$ medium, $w =$ weak, $br =$ broad), and assignment (where appropriate). Proton nuclear magnetic resonance ($^1$H NMR) and carbon nuclear magnetic resonance ($^{13}$C NMR) spectra were obtained on General Electric QE-300 (300 MHz), JEOL JX-400 (400 MHz), or Bruker AM-500 (500 MHz) NMR spectrometers; chemical shifts are expressed in parts per million (δ scale) relative to an internal standard of chloroform ($^1$H, 7.26; $^{13}$C, 77.0), benzene-$d_6$ ($^1$H, 7.20; $^{13}$C, 128.0), or dimethyl sulfoxide-$d_5$ ($^1$H, 2.49; $^{13}$C, 39.5). Data are represented as follows: chemical shift, multiplicity ($s =$ singlet, $d =$ doublet, $t =$ triplet, $q =$ quartet, $m =$ multiplet, $br s =$ broad singlet, $ABq =$ AB quartet), integration, coupling constant in hertz (Hz), and assignment. High resolution mass spectra (HRMS) were obtained at the University of California, Riverside, Mass Spectrometry Facility or at the University of Nebraska, Lincoln, Midwest Center for Mass Spectrometry (with partial support by the National Science Foundation, Biology Division, Grant DIR9017262). High pressure liquid chromatography (HPLC) purification was carried out on a Waters Delta Prep 3000 instrument equipped with a Waters 994 programmable photodiode array detector using Vydac analytical (10 µm, 0.46 x 25 cm) and preparative (10 µm, 2.2 x 25 cm) reverse-phase C18 (201HS) columns. UV spectra were obtained on a Shimadzu UV160U spectrophotometer.

**Reagents and Materials for DNA Studies.** All reaction solutions were prepared with ultrapure water, obtained from a Millipore Milli-Q Plus water purification system. Sonicated, deproteinized calf thymus DNA was purchased from Pharmacia and was dissolved in water to a concentration of 1 mM base pairs (bp) in DNA. Plasmid pBR322 was purchased from Boehringer-Mannheim. Enzymes were obtained from
Boehringer-Mannheim and New England Biolabs and were used with the supplied buffers, with the exception of Klenow enzyme, which was used with HaeIII buffer. Deoxyadenosine 5'-[α-32P]triphosphate and adenosine 5'-[γ-32P]triphosphate were purchased from Amersham or NEN. Other reagents were used as received. Standard techniques were employed for DNA manipulations.34

**DNA Fragment Preparation.** Plasmid pBR322 was linearized with EcoRI restriction endonuclease and then was labeled on the 3' terminus with [α-32P]dATP using the Klenow fragment of DNA polymerase I. Following removal of the 5'-phosphate with calf alkaline phosphatase (CAP), a separate batch of EcoRI-digested plasmid was labeled at the 5' end with [γ-32P]ATP using T4 polynucleotide kinase. After labeling, the DNA was digested with RsaI to yield two end-labeled restriction fragments. The 517 bp fragment was excised from a 5% non-denaturing polyacrylamide gel (17.0 × 15.0 cm × 3 mm thickness) and further isolated according to standard procedures.34 Chemical-sequencing reactions were performed according to published methods.23,24 A molecular weight standard for the non-denaturing gels was prepared as follows. Equal amounts of the 3'-radiolabeled 517 bp fragment and 0.25 µg of pBR322 were combined and digested individually with restriction endonucleases SspI, BspHI, and XmnI. The reaction mixtures were ethanol precipitated, counted, diluted to the same concentration [counts per minute (cpm) per microliter], and then combined in equal volumes with the same cpm of intact 517 bp fragment.

**Footprinting and Cleavage Reactions.** Reactions were executed in a total volume of 20 µL with final concentrations of each component as noted. The conjugate 9 was added to solutions of radiolabeled restriction fragment (~18,000 cpm), calf thymus DNA (100 µM bp), Tris-acetate buffer (25 mM, pH 7.0), and NaCl (10 mM), and the resulting solutions were incubated for 15 min at 37 °C. Footprinting reactions were initiated by addition of MPE•Fe(II) solution (4 µM, prepared by mixing 20 µL of a 100 µM MPE solution with 20 µL of a freshly made, 100 µM ferrous ammonium sulfate solution
and then diluting to 100 µL with water)\textsuperscript{35} and dithiothreitol (4 mM) and proceeded for 9 min at 37 °C. Cleavage reactions were incubated at 37 °C for various defined lengths of time, up to 70 h. All reactions were terminated by freezing (dry ice), and the frozen solution was lyophilized on a Savant rotary speed-vac. The reaction mixtures were resuspended in 80% denaturing formamide loading buffer (4 µL), assayed for radioactivity (Beckman LS 6000SC scintillation counter), and electrophoresed on 8% polyacrylamide denaturing gels (5% crosslink, 7 M urea; 31 x 38.5 cm x 0.4 mm thickness) at 1700 V for 1.5 h. The gels were dried and analyzed using standard phosphor technology. Dried gels were also exposed to preflashed X-ray film at -80 °C with an intensifying screen.

**Quantitative MPE•Fe(II) Footprint Titrations.** Reaction mixtures (20 µL total volume) consisted of the following final concentrations: serially diluted conjugate 9 (500 µM to 1 nM) or distamycin analog 25 (100 µM to 0.1 nM), 3'-labeled 517 bp restriction fragment (20,000 cpm), calf thymus DNA (100 µM bp), Tris-acetate buffer (25 mM, pH 7.0), and NaCl (10 mM). After incubation at 37 °C for 15 min, freshly prepared MPE•Fe(II) solution (2 µM) and dithiothreitol (2 mM) were added to initiate the footprinting reactions. After the reactions proceeded for 9 min at 37 °C, the mixtures were frozen (dry ice) and dried by speed-vac. Reaction mixtures were electrophoresed and analyzed as described above.

**Calculation of Relative Binding Affinities.** Binding isotherms were created following published methods.\textsuperscript{26} Briefly, standard phosphor-imaging technology was used to analyze both binding sites (5'-TTTTT-3' and 5'-AATAA-3') and a reference site (5'-CGTCA-3') by performing volume integrations. The apparent fraction of DNA bound (θ\textsubscript{app}) was calculated and then fit to a Langmuir binding titration isotherm over the range of substrate concentrations. Binding affinities were determined from averaged θ\textsubscript{app} values from three different acceptable (according to the χ\textsuperscript{2} criterion)\textsuperscript{36} gels. Values from the two binding sites were then compared for conjugate 9 and the distamycin analog 25.
Cleavage Trials under Non-denaturing Conditions. Reactions were executed in a total volume of 20 µL with the final concentrations of each component as follows, coupled product 9 (20 or 10 µM), 3'-labeled 517 bp restriction fragment (8,000 cpm), calf thymus DNA (100 µM bp), Tris-acetate buffer (25 mM, pH 7.0), and NaCl (10 mM), and incubated at 37 °C for 60-70 h. Reactions were terminated by ethanol precipitation, and mixtures were dried on a rotary speed-vac. The dried pellet was resuspended in water (10 µL) and Ficoll (6x) loading buffer (2 µL), and the resulting suspension was assayed for radioactivity. Samples were electrophoresed on an 8% non-denaturing polyacrylamide gel (125 V, 17.0 × 15.0 cm × 1.5 mm thickness) positioned behind a cooling fan until the bromophenol blue dye had migrated within 1–2 cm of the bottom of the gel. The gel was then exposed to preflashed X-ray film at −80 °C with an intensifying screen.

Quantitation by Storage Phosphor Technology Autoradiography. Photostimulatable storage phosphor-imaging plates (Kodak S0230) were pressed flat against dried gels and exposed in the dark at 23 °C for 12–18 h. Data from storage plates were obtained from a Molecular Dynamics 400S PhosphorImager. Data analysis was performed by calculation of volume integrations of all bands using the ImageQuant v.3.1 software running on an AST Premium 386/33 computer.
tert-Butyldimethylsilyl chloride (0.895 g, 5.95 mmol, 1.1 equiv) was added to a solution of alcohol 10 (1.26 g, 5.41 mmol, 1 equiv)\(^4\) and triethylamine (1.14 mL, 8.12 mmol, 1.5 equiv) in dichloromethane (100 mL) at 0 °C. After 5 min, the reaction mixture was warmed to 23 °C, 4-dimethylaminopyridine (0.179 g, 1.46 mmol, 0.27 equiv) was added, and the resulting solution was stirred for 17 h. The reaction mixture was then poured into water (100 mL), and the product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 × 150 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The residue was purified by flash chromatography (5% ethyl acetate–hexanes) to yield the (Z)-bromoenyne 11 (1.76 g, 94%) as a yellow oil.

\(^{1}H\) NMR (300 MHz, CDCl\(_3\)), δ:

- 6.38 (t, 1 H, \(J = 2.0\) Hz, C=CH),
- 4.31 (d, 2 H, \(J = 2.0\) Hz, SiOCH\(_2\)),
- 0.92 (s, 9H, SiC(CH\(_3\))\(_3\)),
- 0.24 (s, 9H, Si(CH\(_3\))\(_3\)),
- 0.10 (s, 6H, Si(CH\(_3\))\(_2\))

\(^{13}C\) NMR (125 MHz, CDCl\(_3\)), δ:

- 137.2 (CH=CB\(_r\)),
- 108.8 (CH=CB\(_r\)),
- 101.2 (C=\(\equiv\)),
- 101.1 (C=\(\equiv\)),
- 67.5 (CH\(_2\)OTBS),
- 25.7 (SiC(CH\(_3\))\(_3\)),
- 22.6 (SiC(CH\(_3\))\(_3\)),
- -0.2 (Si(CH\(_3\))\(_3\)),
- -5.5 (Si(CH\(_3\))\(_2\))

FTIR (neat), cm\(^{-1}\):

- 2956 (s), 2858 (m), 2145 (m, C≡C), 1471 (m),
- 1375 (m), 1251 (s), 1132 (s), 1092 (s), 1032 (m),
- 842(s), 779(m)

HRMS (CI):

Calc'd for C\(_{14}\)H\(_{28}\)BrOSi\(_2\) [M + H]\(^+\): 347.0863

Found: 347.0849

TLC (20% EtOAc in hexanes), \(R_f\):

0.89
A solution of the (Z)-bromoenyne 11 (1.76 g, 5.07 mmol, 1 equiv), propargyl alcohol (0.58 mL, 10.1 mmol, 2.0 equiv), and n-propylamine (1.67 mL, 20.3 mmol, 4.0 equiv) in tetrahydrofuran (50 mL) was deoxygenated at 23 °C and then was cooled in ice. Cuprous iodide (0.145 g, 0.760 mmol, 0.15 equiv) and bis(triphenylphosphine)palladium(II) chloride (0.178 g, 0.254 mmol, 0.05 equiv) were added sequentially to the reaction mixture, deoxygenating thoroughly after each addition. Upon completed addition, the reaction mixture was warmed from 0 to 23 °C and then was stirred for 15 h. The reaction mixture was poured into a 1:1 mixture of saturated aqueous ammonium chloride solution and saturated aqueous potassium carbonate solution (60 mL). The product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 x 100 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The residue was purified by flash chromatography (10% ethyl acetate–hexanes) to give the (Z)-enediyne 12 (1.31 g, 80%) as an oil.

\[ \text{1H NMR (500 MHz, CDCl}_3, \delta:} \]
6.06 (t, 1H, \( J = 2.1 \text{ Hz, C=CH} \)), 4.46 (s, 2H, C=CH\text{CH}_2\text{OH}), 4.20 (d, 2H, \( J = 2.1 \text{ Hz, SiOCH}_2\text{R} \)), 0.91 (s, 9H, SiC(CH\text{)}_3\text{)}, 0.22 (s, 9H, Si(CH\text{)}_3\text{)}, 0.07 (s, 6H, Si(CH\text{)}_2\text{)}

\[ \text{13C NMR (125 MHz, CDCl}_3, \delta:} \]
135.0 (RCH=C), 113.3 (RCH=C), 102.4 (C=CTMS), 101.7 (C=CTMS), 95.5 (C=CH\text{CH}_2\text{OH}), 82.1 (C=CH\text{CH}_2\text{OH}), 64.5 (RCH\text{2OTBS}), 51.6 (RCH\text{2OH}), 25.8
FTIR (neat), cm⁻¹: 3358 (br, OH), 2956 (s), 2858 (m), 2134 (m, C=\(\text{C}\)), 1469 (w), 1252 (s), 1120 (s), 1034 (w), 851 (s), 778 (m)

HRMS (CI): Calc’d for C\(_{17}\)H\(_{31}\)O\(_2\)Si\(_2\) [M + H]\(+\): 323.1862

Found: 323.1859

TLC (20% EtOAc in hexanes), \(R_f\): 0.38
Aqueous sodium hydroxide solution (50% w/w, total volume 0.25 mL) was added in five portions over a 2 h period to an ice-cooled solution of the (Z)-enediyne 12 (0.650 g, 2.02 mmol, 1 equiv) in methanol (25 mL). The reaction mixture was then poured into water (100 mL), and the product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 × 100 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The residue was purified by flash chromatography (20% ethyl acetate–hexanes) to provide the (Z)-enediyne 13 as an oil (0.45 g, 89%).

\[ \text{\textsuperscript{1}H NMR (500 MHz, CDCl\_3), } \delta: \]

- 6.04 (dd, 1H, \( J = 4.5 \text{ Hz}, 2.1 \text{ Hz}, \text{C}=\text{CH} \)), 4.47 (d, 2H, \( J = 6.0 \text{ Hz}, \text{RCH}_2\text{OH} \)), 4.22 (d, 2H, \( J = 1.5 \text{ Hz}, \text{SiOCH}_2 \)), 3.29 (m, 1H, \( \text{C}=\text{CH} \)), 1.62 (t, 1H, \( J = 6.1 \text{ Hz}, \text{RCH}_2\text{OH} \)), 0.92 (s, 9H, \( \text{SiC(CH}_3)_3 \)), 0.08 (s, 6H, \( \text{Si(CH}_3)_2 \))

\[ \text{\textsuperscript{13}C NMR (125 MHz, CDCl\_3), } \delta: \]

- 135.6 (\( \text{RCH}=\text{C} \)), 112.0 (\( \text{RCH}=\text{C} \)), 95.5 (\( \text{C}=\text{CCH}_2\text{OH} \)), 83.6 (\( \text{C}=\text{CH} \)), 81.0 (\( \text{C}=\text{CH} \)), 64.6 (\( \text{RCH}_2\text{OTBS} \)), 51.6 (\( \text{RCH}_2\text{OH} \)), 25.8 (\( \text{SiC(CH}_3)_3 \)), 18.3 (\( \text{SiC(CH}_3)_3 \)), -5.5 (\( \text{Si(CH}_3)_2 \))

\[ \text{FTIR (neat), cm}^{-1}: \]

- 3289 (br, OH, and s, \( \text{C}=\text{CH} \)), 2932 (s), 2858 (s), 2217 (w), 2103 (w), 1467 (m), 1257 (s), 1118 (s), 1032 (m), 840 (s), 780 (s)

\[ \text{HRMS (FAB):} \]

- Calc'd for C\(_{14}\)H\(_{22}\)NaO\(_2\)Si [M + Na]^+: 273.1287
Found: 273.1284

TLC (20% EtOAc in hexanes), $R_f$: 0.29
Methanesulfonyl chloride (0.17 mL, 2.3 mmol, 3.0 equiv) was added dropwise over 10 min to an ice-cooled solution of the (Z)-enediyne 13 (0.19 g, 0.75 mmol, 1 equiv) and triethylamine (0.55 mL, 3.8 mmol, 5.0 equiv) in dichloromethane (8 mL). The resulting suspension was stirred for 15 min at 0 °C. A solution of anhydrous hydrazine in methanol (1:1 v/v, 1.5 mL, 23.9 mmol hydrazine, 31.8 equiv) was then added directly by syringe, and the reaction mixture was stirred at 0 °C for 18 h. The reaction solution was poured into half-saturated brine (100 mL), and the product alkylhydrazine was extracted with 5% methanol in dichloromethane (2 × 100 mL). The combined organics were washed with brine (100 mL), dried over sodium sulfate, and concentrated in vacuo.

The crude alkylhydrazine (theory, 0.75 mmol, 1 equiv) was taken up in diethyl ether (70 mL), and the resulting solution was deoxygenated at 23 °C and then was cooled to −78 °C. A deoxygenated solution of 4-methyl-1,2,4-triazoline-3,5-dione (MTAD, 0.170 g, 1.5 mmol, 2.0 equiv) in diethyl ether (10 mL) was added quickly to the reaction mixture via cannula. The resulting pink solution was stirred at −78 °C for 15 min and was then poured into half-saturated brine (150 mL). The product allene was extracted with pentane (3 × 200 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The residue was purified by flash chromatography (5% ethyl acetate–hexanes), affording the allene 14 (70.0 mg, 40%) as a yellow oil.

^1H NMR (400 MHz, C_6D_6), δ:

- 6.86 (t, 1H, J = 6.8 Hz, HC=C=CH₂),
- 6.08 (s, 1H, C=CH),
- 4.69 (d, 2H, J = 6.8 Hz, HC=C=CH₂),
- 4.35 (s, 2H, SiOCH₂),
- 2.98 (s,
1H, C=CH), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.01 (s, 6H, Si(CH$_3$)$_2$)

$^{13}$C NMR (100 MHz, C$_2$D$_2$), $\delta$: 210.5 (C=C=C), 146.5 (RCH=C), 104.2 (RCH=C), 90.8 (C=C=CH$_2$), 84.6 (C=CH), 81.0 (C=CH), 78.3 (C=C=CH$_2$), 62.9 (RCH$_2$OTBS), 26.0 (SiC(CH$_3$)$_3$), 18.4 (SiC(CH$_3$)$_3$), -5.5 (Si(CH$_3$)$_2$)

FTIR (neat), cm$^{-1}$: 3310 (m), 2955 (s), 2929 (s), 2857 (s), 2100 (w), 1934 (m), 1666 (w), 1604 (w), 1464 (m), 1390 (w), 1362 (w), 1255 (s), 1192 (m), 1118 (s), 1076 (m), 1006 (w), 939 (w), 841 (s), 778 (s), 671 (w), 638 (w)

HRMS (CI): Calc'd for C$_{14}$H$_{23}$OSi [M + H]$^+$: 235.1518

TLC (50% EtOAc in hexanes), $R_f$: 0.93

Found: 235.1516
A total volume of 1.75 mL of triethylamine trihydrofluoride was added in four portions over a period of 2 h to a solution of the allene 14 (70.0 mg, 299 µmol, 1 equiv) in dichloromethane (10 mL) at 23 °C with periodic monitoring of the progress of the reaction by thin-layer chromatography. After being stirred for an additional 1 h, the reaction mixture was poured into water (100 mL), and the product was extracted with dichloromethane (3 × 100 mL). The combined organics were dried over sodium sulfate and were concentrated. The residue was purified by flash chromatography (30% ethyl acetate–hexanes), providing the alcohol 15 as a (volatile) yellow oil (27.3 mg, 76%).

\( ^1H\) NMR (500 MHz, \( C_6D_6 \), \( \delta \): 6.76 (t, 1H, \( J = 6.7 \text{ Hz} \), H\( C=C=CH_2 \)), 5.69 (s, 1H, C\( =CH \)), 4.64 (d, 2H, \( J = 6.7 \text{ Hz} \), H\( C=C=CH_2 \)), 3.93 (d, 2H, \( J = 5.6 \text{ Hz} \), H\( OCH_2 \)), 2.96 (s, 1H, C\( =CH \)), 0.70 (t, 1H, \( J = 5.8 \text{ Hz} \), H\( OCH_2 \))

\( ^13C\) NMR (125 MHz, DMSO-\( d_6 \), \( \delta \): 209.8 (C\( =C=C \)), 147.4 (RCH\( =C \)), 87.0 (RCH\( =C \)), 87.0 (C\( =C=CH_2 \)), 80.6 (RC\( =CH \)), 79.1 (RC\( =CH \)), 75.6 (C\( =C=CH_2 \)), 60.5 (CH\( _2OH \))

FTIR (neat), cm\(^{-1}\): 3294 (br, OH, and s, C\( =C \)), 2924 (s), 2092 (w), 1932 (s, C\( =C=C \)), 1718 (m), 1600 (w), 1385 (m), 1292 (m), 1056 (s), 852 (s)

HRMS (EI): Calc'd for C\(_8\)H\(_8\)O [M]\(^+\): 120.0575
Found: 120.0572

TLC (30\% EtOAc in hexanes), \( R_f \): 0.44
Fumaric acid (0.200 g, 1.72 mmol, 1 equiv), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 0.992 g, 5.16 mmol, 3.0 equiv), and 4-dimethylaminopyridine (7.5 mg, 61 µmol, 0.04 equiv) were added sequentially to a solution of pentafluorophenol (0.955 g, 5.19 mmol, 3.0 equiv) in dichloromethane (50 mL). After being stirred at 23 °C for 12 h, the reaction mixture was poured into water (100 mL) and the product was extracted with dichloromethane (3 × 100 mL). The combined organic layers were dried over sodium sulfate and were concentrated to a volume of 100 mL. The concentrated product solution was extracted with saturated aqueous sodium bicarbonate solution (3 × 90 mL), was dried over sodium sulfate, and was concentrated. Upon addition of cold dichloromethane (−20 °C, 10 mL), the product precipitated as a pale yellow solid. The solid was collected, and the filtrate was concentrated. The residue upon concentration was precipitated with cold dichloromethane, as before, and the precipitate was collected and combined with the first crop of product. The solid product was dried under vacuum to provide 0.489 g (63%) of bis(pentafluorophenyl) ester 20 (mp 161–163.5 °C dec).

\[ \text{1}^1\text{H NMR (400 MHz, CDCl}_3\text{), } \delta: \]
7.34 (s, 2H, CH=CH)

\[ \text{13}^\text{C NMR (100 MHz, CDCl}_3\text{), } \delta: \]
160.2 (CO₂R), 134.0 (C(O)CH=CHC(O)), 124–143 (complex, pentafluorophenyl carbons)

\[ \text{FTIR (neat), } \text{cm}^{-1}: \]
3097 (w), 1769 (m), 1516 (s), 1324 (w), 1282 (s), 1120 (s), 1026 (w), 1002 (s), 907 (w)
HRMS (FAB): Calc'd for $\text{C}_{16}\text{H}_{2}\text{F}_{10}\text{O}_{4}$ [M]: 447.9793

TLC (0.4% AcOH in EtOAc), $R_f$: 0.94

Found: 447.9823
Bis(pentafluorophenyl) ester 20 (0.286 g, 0.638 mmol, 3.0 equiv) and 4-dimethylaminopyridine (~2 mg, 20 µmol, 0.01 equiv) were added sequentially to a pale yellow solution of alcohol 15 (25 mg, 210 µmol, 1 equiv) and triethylamine (148 µL, 1.07 mmol, 5.0 equiv) in dichloromethane (25 mL) at 23 °C. After stirring for 3.5 h at 23 °C, the reaction mixture was poured into water (90 mL), and the product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 x 100 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The residue was purified by flash chromatography (20% toluene–hexanes) to provide 48 mg (60%) of pentafluorophenyl ester 21 as an oil.

\[ ^1H \text{ NMR (400 MHz, CDCl}_3\text{), } \delta: \]
\[ 7.11 \text{ (ABq, 2H, } J = 15.9 \text{ Hz, } \Delta v = 18.9 \text{ Hz,} \]
\[ \text{C(O)CH=CHC(O)}, \ 6.54 \text{ (t, 1H, } J = 7.2 \text{ Hz,} \]
\[ \text{HC=C=CH}_2\text{), 5.59 (s, 1H, } \text{RCH=C), 5.13 (d,} \]
\[ 2\text{H, } J = 6.8 \text{ Hz, } \text{C=C=CH}_2\text{), 4.86 (s, 2H,} \]
\[ \text{CH}_2\text{OR), 3.36 (d, 1H, } J = 2.4 \text{ Hz, } \text{C=CH)} \]

\[ ^{13}C \text{ NMR (100 MHz, CDCl}_3\text{), } \delta: \]
\[ 211.0 \text{ (C=C=C), 163.4 (CO}_2\text{CH}_2\text{), 160.6} \]
\[ \text{(CO}_2\text{C}_6\text{F}_6\text{), 141.1 (RCH=C), 137.0 and 130.4} \]
\[ \text{(C(O)CH=CHC(O)), 107.6 (RCH=C), 90.9} \]
\[ \text{(C=C=CH}_2\text{), 85.5 (RC=CH), 79.5 (RC=CH),} \]
\[ 79.2 \text{ (C=C=CH}_2\text{), 65.0 (CH}_2\text{OR), 124–143} \]
\[ \text{(complex, pentafluorophenyl carbons) } \]

\[ \text{FTIR (neat), cm}^{-1}: \]
\[ 3294 \text{ (m), 3077 (w), 2995 (w), 2933 (w), 2667} \]
(w), 2462 (w), 2092 (w), 1933 (m), 1773 (s), 1732 (s), 1645 (m), 1604 (w), 1522 (s), 1471 (w), 1376 (w), 1285 (s), 1201 (s), 1146 (m), 1121 (s), 1005 (s), 856 (m), 760 (m)

HRMS (FAB):
Calc'd for C_{18}H_{9}F_{5}O_{4} [M]^-: 384.0421
Found: 384.0445

TLC (20% EtOAc in hexanes), R_f: 0.67
Palladium (10%) on activated carbon (11.6 mg) was added to a 100 mL Schlenk-type flask (the use of an oversized flask appears to be important for success in this reaction) containing a deoxygenated solution of 4-nitropyrrrole-2-carboxamide 16 (33.0 mg, 66.3 µmol, 1 equiv)\(^7\) in methanol (8.0 mL), and the resultant suspension was deoxygenated. The reaction flask was then purged with hydrogen gas, and the reaction solution was stirred under 1 atm of hydrogen for 2.5 h at 23 °C. The reaction mixture was filtered through Celite, and the filtrate was concentrated. The residue was concentrated twice from dichloromethane (10 mL) and was then lyophilized from benzene (10 mL) to afford the 4-aminopyrrole-2-carboxamide 17 (\(R_f = 0.30\), 3% ammonium hydroxide-methanol) as an air-sensitive yellow powder that was immediately coupled with the pentafluorophenyl ester 21.

Pentafluorophenyl ester 21 (25.5 mg, 66.3 µmol, 1.0 equiv) was added to a deoxygenated solution of the crude 4-aminopyrrole-2-carboxamide 17, prepared above, in \(N,N\)-dimethylformamide (6.0 mL). The reaction mixture was observed to darken to a golden color. After being stirred for 1 h at 23 °C, the reaction mixture was poured into water (65 mL), and the product was extracted with dichloromethane (3 \(\times\) 60 mL). The combined organic layers were washed with water (2 \(\times\) 100 mL), were dried over sodium...
sulfate, and were concentrated. The residue was purified by flash chromatography (1% ammonium hydroxide in 1:1 acetonitrile–methanol) wherein acetic acid (2–3 drops) was added to each fraction as it eluted. Fractions containing product 9 were pooled and concentrated, and the residue was lyophilized from dimethyl sulfoxide (2 mL) to afford the conjugate 9 as its acetate salt (yellow film, 17 mg, 36%).

$^1$H NMR (400 MHz, DMSO-$d_6$), δ: 10.66 (s, 1H, CH=CHCONH), 9.99 (s, 1H, NH), 9.90 (s, 1H, NH), 8.07 (br s, 1H, CONHCH$_3$), 7.34 (s, 1H, aromatic), 7.24 (s, 1H, aromatic), 7.19 (d, 1H, $J = 14.6$ Hz, CH=CH), 7.17 (s, 1H, aromatic), 7.03 (s, 1H, aromatic), 6.97 (s, 1H, aromatic), 6.81 (s, 1H, aromatic), 6.68 (d, 1H, $J = 15.8$ Hz, CH=CH), 6.41 (t, 1H, $J = 7.0$ Hz, HC=C=CH$_2$), 5.74 (s, 1H, C=CHC≡C), 5.30 (d, 2H, $J = 7.0$ Hz, HC=C=CH$_2$), 4.77 (s, 2H, CH$_2$OR), 4.48 (s, 1H, C≡CH), 3.86 (s, 3H, NCH$_3$), 3.83 (s, 3H, NCH$_3$), 3.78 (s, 3H, NCH$_3$), 3.17 (m, 2H, CONHCH$_2$), 2.23 (t, 2H, $J = 6.6$ Hz, CH$_2$N(CH$_3$)$_2$), 2.12 (s, 6H, N(CH$_3$)$_2$), 1.82 (s, 3H, acetate), 1.60 (m, 2H, CH$_2$CH$_2$CH$_2$N(CH$_3$)$_2$)

$^{13}$C NMR (100 MHz, DMSO-$d_6$), δ: 210.4 (C≡C=C), 172.5 (°COCH$_3$), 164.5 (RCO$_2$CH$_2$R), 161.2 (CONH), 159.5 (CONH), 158.4 (CONH), 158.2 (CONH), 141.2 (HC≡CCH≡C), 137.8 (C(O)CH=CHC(O)), 127.7 (C(O)CH=CHC(O)), 123.3, 123.0, 122.8, 122.1, 122.0, 121.3, 118.8, 118.4, 117.7,
FTIR (neat), cm\(^{-1}\):

3401 (br), 3284 (br), 2943 (m), 1931 (w), 1720 (m), 1641 (s), 1578 (s), 1555 (s), 1465 (m), 1435 (s), 1405 (s), 1349 (w), 1290 (m), 1263 (s), 1208 (m), 1165 (m), 1149 (m), 1106 (w), 1049 (w), 1020 (m), 1005 (m), 885 (w), 814 (w), 774 (m), 668 (w), 609 (w)

HRMS (FAB):

Calc'd for C\(_{35}H_{40}N_8O_6\) [M + H]: 669.3149

Found: 669.3142

TLC (3% NH\(_4\)OH in CH\(_3\)OH), \(R_f\):

0.38
Thermal cyclization studies of conjugate 9 (3.0–3.8 mM) were conducted in dimethyl sulfoxide-$d_6$ solution containing 1,4-cyclohexadiene (CHD, 30–300 mM) in sealed NMR tubes. Each reaction solution was thoroughly deoxygenated by several freeze–pump–thaw cycles prior to flame-sealing under vacuum. Samples prepared for kinetics runs contained N,N-dimethylformamide (25 mM) as an internal standard and were analyzed periodically by $^1$H NMR, integrating against the formyl proton resonance. Thermolyses were conducted at temperatures from 30–37 °C in a regulated sand bath for a total reaction period of 85–90 h. Upon the completion of the reaction, the product solutions were analyzed by $^1$H NMR and by reverse-phase high-performance liquid chromatography (rp-HPLC). rp-HPLC was conducted using Vydac analytical (10 µm, 0.46 × 25 cm, flow rate of 0.4 mL/min) or preparative (10 µm, 2.2 × 25 cm, flow rate of 7.0 mL/min) reverse-phase C18 (201HS) columns, employing acetonitrile and aqueous ammonium acetate buffer solution (10 mM, pH 5.5) as eluent in a linear gradient of 30:70 to 60:40, respectively, over a 30 min period followed by isocratic elution (60:40). Cyclization product 22 was typically formed in 25% yield. Isomers 23 and 24 were also formed in 25% (combined) yield. Product 22 (retention time of 25 min) and the mixture of isomers 23 and 24 (inseparable, retention time of 37 min) were separated by HPLC and were characterized as follows.
Cyclization product 22:

$^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$: 10.65 (s, 1H, CH=C\(\text{CH}(O)\text{NH}\)), 9.99 (s, 1H, NH), 9.89 (s, 1H, NH), 9.07 (br s, 1H, NH\(\text{CH}_2\)), 7.33 (s, 1H, aromatic), 7.15–7.30 (5H, aromatic + CH=CH), 7.23 (s, 1H, aromatic), 7.17 (s, 1H, aromatic), 7.02 (s, 1H, aromatic), 6.96 (s, 1H, aromatic), 6.81 (s, 1H, aromatic), 6.69 (d, 1H, $J = 15.0$ Hz, CH=CH), 5.18 (s, 2H, CH$_2$OR), 3.85 (s, 3H, NCH$_3$), 3.83 (s, 3H, NCH$_3$), 3.78 (s, 3H, NCH$_3$), 3.18 (m, 2H, C(O)NH\(\text{CH}_2\)), 2.31 (s, 3H, ArCH$_3$), 2.23 (t, 2H, $J = 7.0$ Hz, CH$_2$N(CH$_3$)$_2$), 2.12 (s, 6H, N(CH$_3$)$_2$), 1.82 (s, acetate), 1.59 (m, 2H, CH$_2$CH$_2$N)

FTIR (neat), cm$^{-1}$: 3384 (br), 2919 (m), 2849 (w), 1725 (w), 1660 (m), 1637 (s), 1584 (s), 1549 (s), 1531 (m), 1467
Isomers 23 and 24:

$^1$H NMR (400 MHz, DMSO-$d_6$), $\delta$: 10.67 (s, 1H, CH=CHC(O)NH), 9.99 (s, 1H, NH), 9.89 (s, 1H, NH), 8.07 (br s, 1H, NHCH$_2$), 7.33 (s, 1H, aromatic), 7.17–7.32 (5H, aromatic + CH=CH), 7.24 (s, 1H, aromatic), 7.17 (s, 1H, aromatic), 7.02 (s, 1H, aromatic), 6.96 (s, 1H, aromatic), 6.81 (s, 1H, aromatic), 6.70 (d, 1H, $J = 15.0$ Hz, CH=CH), 5.55–5.87 (m, cyclohexadienyl protons), 5.20 (s, 2H, 

HRMS (FAB):
Calc'd for C$_{35}$H$_{43}$N$_8$O$_6$ [M + H]$^+$: 671.3305
Found: 671.3297
CH₂OR), 3.85 (s, 3H, NCH₃), 3.83 (s, 3H, NCH₃), 3.78 (s, 3H, NCH₃), 3.18 (m, 2H, NHCH₂R), 2.65 (d, ArCH₂CH), 2.22 (t, 2H, J = 7.0 Hz, CH₂CH₂N(CH₃)₂), 2.12 (s, 6H, N(CH₃)₂), 1.74–1.76 (-OAc (buffer)), 1.59 (m, 2H, CH₂CH₂N(CH₃)₂)

FTIR (neat), cm⁻¹:
3383 (br), 2954 (w), 2908 (s), 2837 (s), 1655 (w), 1631 (m), 1578 (s), 1561 (s), 1543 (m), 1525 (m), 1467 (w), 1402 (m), 1349 (w), 1249 (w), 1061 (br), 861 (m)

HRMS (FAB):
Calc'd for C₄₁H₄₉N₈O₆ [M + H]⁺: 749.3775
Found: 749.3777
Chapter 2

Studies Directed Toward the Total Synthesis of Tetracycline
Introduction

The tetracyclines were discovered 50 years ago as metabolites from assorted Streptomyces strains and proceeded to become some of the most useful broad-spectrum antibiotics on the market. The first tetracycline to be isolated was chlortetracycline 26 (trademarked Aureomycin), followed shortly thereafter by oxytetracycline 27 (trademarked Terramycin). Tetracycline itself (28) was first prepared chemically by the hydrogenolysis of chlortetracycline in 1953 and was subsequently isolated from the fermentation products of Streptomyces aureofaciens.39

The tetracyclines are highly functionalized molecules, containing polar groups, acidic functionality, and as many as six stereogenic centers within an octahydronaphthacene core. They contain two distinct chromophoric regions which provide highly characteristic UV spectra.40 These yellow crystalline compounds also fluoresce, especially when complexed with metal cations. Under mildly acidic conditions (pH ~2–6), epimerization at the C-4 position can occur; however, the natural α-C-4 stereochemistry of tetracycline (see 28 below) can be re-established quantitatively by warming tetracycline mixtures in a basic medium containing a metal cation.41 The binding of a metal cation such as calcium between
the C-4 amino group and the C-12a hydroxyl group is possible only when they are in a cis relationship.

The structures of chlortetracycline and oxytetracycline were elucidated in the early 1950s through extensive degradation studies. This landmark effort was carried out almost exclusively by chemists at Pfizer Inc. in collaboration with R. B. Woodward and co-workers at Harvard University. Results from these degradation studies have influenced the efforts to develop a synthetic route to tetracycline described in this dissertation and are briefly mentioned here. Reduction of chlortetracycline or oxytetracycline with zinc in acetic acid gave rise to loss of the 4-dimethylamino and 12a-hydroxyl groups; alkali-induced rearrangement then provided the substituted phthalides 29 and 30, respectively, by a retro-Dieckmann reaction (eq 1). Similarly, treatment of chlortetracycline or tetracycline with dilute alkali led to their isomerization to form the products 31 and 32, respectively. Under acidic conditions (pH ≤2) tetracyclines are dehydrated to form anhydrotetracyclines (33). The dehydration of oxytetracycline to give 33 (R₁ = H, R₂ = OH) can then lead to its further degradation under basic conditions to provide the apoterramycins (34). These studies indicate the acute sensitivity toward both acid and base of the full tetracycline
system and suggest that planned syntheses should provide for its formation under mild conditions.

Several of the tetracyclines display antimicrobial activity effective against both gram-positive and gram-negative bacteria. Most tetracyclines, including 28, prevent bacterial growth through inhibition of protein synthesis, the result of specific binding of the drug to a ribosomal subunit in the cytoplasm. Some tetracyclines, however, do not directly inhibit bacterial protein synthesis, but rather appear to interfere with the bacterial cell membrane to cause cell lysis. Tetracycline (28) once enjoyed common usage as a broad-spectrum antibiotic; however, the emergence of resistant bacterial strains has curtailed its use in the clinic.

It has been determined from the study of numerous derivatives and degradation products of tetracycline that considerable structural modification of the non-chromophoric regions of rings B, C, and D can be performed without loss of antimicrobial activity. In fact, such changes have produced many new bioactive compounds. A number of these tetracycline analogs have been prepared through chemical modification of the natural tetracyclines, although some have been prepared via laborious total syntheses (vide infra).

Despite all the synthetic and semi-synthetic studies of the tetracyclines, the parent tetracycline (28) has never been prepared through total chemical synthesis. One motivation for such an effort is this finding that chemical modification of tetracycline can overcome bacterial resistance. A concise and versatile laboratory synthesis of 28 would allow for the preparation of antibiotic candidates that are currently unavailable by semi-synthesis and might serve to revitalize interest in this family of antibiotics. This thesis describes efforts to accomplish this objective.

Background

The first total synthesis of a member of the tetracycline class was described by Woodward and co-workers in 1962. 6-Demethyl-6-deoxytetracycline 42, the simplest
biologically active tetracycline which can obtained by the hydrogenolysis of the natural product 6-demethyltetracycline, was prepared in racemic form by the stepwise construction of rings D through A as shown in Scheme IV. Their synthesis is summarized as follows. Claisen condensation of methyl 3-methoxybenzoate with methyl acetate (NaH, DMF) and subsequent alkylation of the crude product with methyl chloroacetate gave an intermediate keto diester in 55% overall yield. Michael addition of this keto diester to methyl acrylate (Triton B, CH₃OH) provided the keto triester 35 (88% crude yield). Decarboxylative hydrolysis of 35 (AcOH, H₂SO₄, H₂O, 100 °C) followed by hydrogenation of the intermediate keto diacid (H₂, Pd/C, AcOH, 200 psi) afforded a diacid in which the conjugated ketone had been deoxygenated. It should be noted that the ketone was removed merely to prepare the simple tetracycline initially targeted. Woodward and co-workers saw this group (which would become the C-6 position of 42) as a way to introduce further functionality in future syntheses of the more complex tetracyclines containing the C-6 methyl and hydroxyl groups (such as 26–28). para-Chlorination (Cl₂, AcOH, 15 °C) of the aromatic ring of the diacid was carried out to block cyclization at this position in the next step. The C ring was then established by internal Friedel–Crafts acylation (liquid hydrogen fluoride, 15 °C) to give the tetralone 36 (19% yield from 35). Conversion of the acid to the methyl ester (H₂SO₄, CH₃OH, 66%) and subsequent Claisen/Dieckmann condensation of this product with dimethyl oxalate (NaH, DMF, CH₃OH, 80–120 °C, 45%) provided the tricyclic compound 37. The latter reaction required much experimentation in order to overcome the starting material’s propensity to undergo an internal condensation. Ester hydrolysis and subsequent decarboxylation of 37 (AcOH, HCl, H₂O, 100 °C, 73%) then provided a 1,2-dione that was condensed with n-butyl glyoxylate in the presence of magnesium methoxide (refluxing toluene, 52%) to give, as a single isomer, the ester 38. Michael addition of dimethylamine to ester 38 (–10 °C) gave an amino ester adduct reversibly; direct reduction of this product with sodium borohydride (wet 1,2-dimethoxyethane, –70 °C) afforded the alcohol 39 in 52% yield.
Reagents and conditions: (a) CH₃CO₂CH₃, NaH, DMF; (b) NaH, ClCH₂CO₂CH₃, 55%; (c) H₂C=CHCO₂CH₃, Triton B, CH₃OH, 88%; (d) AcOH, H₂SO₄, H₂O, 100 °C; (e) H₂, Pd/C, AcOH, 200 psi; (f) Cl₂, AcOH, 15 °C; (g) HF, 15 °C, 19% from 35; (h) H₂SO₄, CH₃OH, 66%; (i) NaH, DMF, CH₃OCCO₂CH₃, CH₃OH, 80→120 °C, 45%; (j) AcOH, HCl, H₂O, 100 °C, 73%; (k) nBu₂CCHO, Mg(OCH₃)₂, PhCH₃, reflux, 52%; (l) HN(CH₃)₂, −10 °C; (m) NaBH₄, H₂O, DME, −70 °C, 52% from 38; (n) p-TsOH, PhCH₃, reflux, 90%; (o) Zn, HCO₂H, 81%; (p) H₂, Pd/C, Et₃N, EtOH, 91%; (q) ClCO₂iPr, Et₃N, CHCl₃, 0 °C; (r) (EtOMg₂, EtO₂CCH₂CONH₂Bu), CH₂CN; (s) NaH, DMF, 23 °C; CH₃OH, 120 °C, 15% from 40; (t) 48% HBr/H₂O, 100 °C, 72%; (u) O₂, CeCl₃, CH₃OH, DMF, pH 10 buffer; (v) CaCl₂, HOCH₂CH₂NH₂ (pH 8.5), nBuOH, H₂O, reflux, 6.5%.
(equilibration in the dimethylamine addition reaction ensured that the side chain would be in the more stable equatorial position). Alcohol 39 then underwent lactonization (p-toluenesulfonic acid, refluxing toluene, 90%), reduction (Zn, HCO₂H, 81%), and dechlorination (H₂, Pd/C, Et₃N, EtOH, 91%) to provide amino acid 40. Formation of a mixed anhydride from 40 (ClCO₂iPr, Et₃N, CHCl₃, 0 °C) allowed for coupling with the ethoxymagnesio derivative of ethyl N-t-butylmalonamate (prepared by reaction of ethyl cyanoacetate with isobutylene in a sulfuric and acetic acid mixture) in acetonitrile. Finally, the closure of the A ring by Dieckmann condensation was effected in a capricious final step to provide the tetracyclic product 41 in 15% overall yield from 40 (NaH, DMF, 23 °C; CH₃OH, 120 °C). Based upon inspection of models and by comparison with the degradation products of 6-demethyltetracycline, Woodward and co-workers proposed that the more stable orientation for the dimethylamino group would be as shown (β-epimer). Treatment of 41 under strongly acidic conditions (48% HBr, H₂O, 100 °C, 72%) led to cleavage of the t-butylamide and methyl ether protective groups. Hydroxylation of the C-12a position using molecular oxygen (CeCl₃, CH₃OH, DMF, pH 10 buffer) and subsequent epimerization of the C-4 dimethylamino group in the presence of calcium chloride (pH 8.5 ethanolamine buffer, n-BuOH, H₂O, reflux) provided the target compound 42 in 6.5% yield. Although a remarkable feat, their synthesis provided 42 in 22 steps with an overall yield of only ~0.003%.

One of the most important aspects of the Woodward synthesis is the C-12a hydroxylation reaction. The oxygenation conditions utilized in the synthesis of 42 were based on the group’s previous work with tetracycline analogs in which the use of molecular oxygen in the presence of a variety of metal ions was found to effect the desired transformation. However, this reaction, when conducted on the authentic substrate, provided the desired hydroxylation product in very low yields, albeit stereoselectively. Woodward proposed that this selectivity arose from the orientation of the dimethylamino group, which effectively blocked oxygenation on the β-face.
A different synthesis of (±)-6-demethyl-6-deoxytetracycline 42 was published shortly afterwards by Muxfeldt and co-workers.\textsuperscript{52} Their approach to 42 was also designed for later adaptation to produce the more highly substituted and synthetically challenging tetracycline, oxytetracycline (27).\textsuperscript{53} As established by degradation chemistry, the more functionalized tetracyclines are extremely sensitive to acid and base, and, as discussed above, the C-6 hydroxyl group is susceptible to dehydration under acidic conditions (see 33). Under basic conditions a retro-Dieckmann reaction occurs to form apoterramycin (see 34). Muxfeldt and co-workers circumvented these problems by protecting the three hydroxyl groups of 27 with base-stable protecting groups that could later be cleaved under mildly acidic conditions.

The Muxfeldt synthesis began with the protection of juglone as its acetate (Ac\textsubscript{2}O, 83\%) and subsequent Diels–Alder reaction of this product with 1-acetoxy-1,3-butadiene (refluxing benzene, 60\%) to provide the endo-adduct 43 in racemic form (Scheme V). This reaction established what would become the C-6 and C-5a stereocenters. Stereoselective addition of methylmagnesium iodide (toluene, –70 °C, 82\%) to 43 was then followed by saponification and concomitant epimerization to give the trans ring fusion (KOH, H\textsubscript{2}O, 84\%). Protection of the resulting 1,3-diol as its acetonide (acetone, CuSO\textsubscript{4}, 84\%) and the phenol as its acetate ester (Ac\textsubscript{2}O, NaOAc, 100 °C, 95\%) led to the tricyclic structure 44. Dihydroxylation of 44 under standard conditions (OsO\textsubscript{4}, KClO\textsubscript{3}, THF, H\textsubscript{2}O, 50 °C, 89\%) provided a diol, which was cleaved to the dialdehyde with lead(IV) acetate (acetone, 40 °C) followed by an intramolecular aldol dehydration reaction (DABCO, piperidine, AcOH, xylene, reflux) to provide the ring-contracted product 45 in 52\% overall yield. Ozonolysis of 45 was followed by treatment with water to afford a dialdehyde in 68\% yield. Cleavage of the extraneous carbonyl group and hydrolysis of the phenyl ester was brought about under basic conditions (Na\textsubscript{2}CO\textsubscript{3}, CH\textsubscript{2}Cl\textsubscript{2}, 85\%) to give a mixture of aldehydes. This mixture was then submitted to the following series of reactions: enamine formation with piperidine (refluxing benzene, 91\%), protection of the phenol (NaH, THF,
Reagents and conditions: (a) Ac₂O, 83%; (b) 1-acetoxy-1,3-butadiene, PhH, reflux, 60%; (c) CH₃MgI, PhCH₃, -70 °C, 82%; (d) KOH, H₂O, 84%; (e) acetone, CuSO₄, 84%; (f) Ac₂O, NaOAc, 100 °C, 95%; (g) OsO₄, KClO₃, THF, H₂O, 50 °C, 89%; (h) Pb(OAc)₄, acetone, 40 °C; (i) DABCO, piperidine, AcOH, xylene, reflux, 52%; (j) O₃, CHCl₃, -50 °C; (k) H₂O, 68%; (l) Na₂CO₃, CH₂Cl₂, 85%; (m) piperidine, PhH, reflux, 91%; (n) NaH, THF, 0 °C; ClCH₂OCH₃, 23 °C, 90%; (o) deactivated silica gel, 70–80%; (p) 2-phenylthiazolin-5-one, basic lead acetate, THF, 20 °C, 70%; (q) (methyl 3-oxoglutaramate, nBuLi), THF, -78→25 °C; LiOrBu, reflux, 27%; (r) AcOH, H₂O, reflux, 90%; (s) O₂, NaH, P(0Et)₃, THF, DMF, H₂O; (t) HCl, CH₃OH, 47%; (u) CH₃I, THF; HCl, H₂O; (v) dimethyl sulfate, DIPEA, THF, EtOH, 23%. 

Scheme V
0 °C, chloromethyl methyl ether, 23 °C, 90%), and enamine cleavage with moist silica gel to give a solution of the aldehyde 46 in crude form. The latter product possessed the desired C-5 stereochemistry and was one of the key intermediates in Muxfeldt's strategy, embodying the left-hand half of the target tetracycline. The remaining two key components in the Muxfeldt synthesis were added sequentially in the next two steps. Thus, aldehyde 46 was condensed with 2-phenylthiazolin-5-one in the presence of basic lead acetate\(^\text{54}\) to provide thiazolone 47 (70%). Then, in a truly remarkable reaction, three C–C bonds were formed to complete the synthesis of the tetracyclic framework. Thus, reaction of 47 with the lithium salt of methyl 3-oxoglutaramate (\(n\)-BuLi, \(-78\rightarrow25 \, ^\circ\mathrm{C}\)) effected closure of the A ring as shown in the partial structure 48. Further addition of base (LiOr-Bu) and heating of the reaction mixture to reflux provided, after separation of the diastereomers by crystallization, the tetracyclic compound 49 in 27% yield. Deprotection of the aromatic ether (AcOH, H\(_2\)O, reflux, 90%) preceded the critical C-12a hydroxylation reaction. Muxfeldt and co-workers conducted the latter transformation using gaseous oxygen in the presence of triethylphosphite and sodium hydride (THF, DMF, H\(_2\)O). The crude C-12a-hydroxylated product was formed in ~52% yield along with the C-11a-hydroxylated product (~14% yield). This crude mixture was treated with acid (HCl, CH\(_3\)OH) to afford the tetracycline 50 in 47% yield. Even with the C-4 dimethylamino group existing as the desired \(\alpha\)-epimer, the C-12a hydroxylation appeared to take place largely with the desired \(\alpha\)-stereochemistry. Methylation and hydrolysis of the thioamide protecting group (CH\(_3\)I, THF; HCl, H\(_2\)O) liberated the amino group, which was methylated under standard conditions (dimethyl sulfate, DIPEA, THF, EtOH) to provide oxytetracycline 27 in 23% yield. In this manner, oxytetracycline 27 was synthesized in 22 linear steps in an overall yield of ~0.1%. The key feature of this work is that three key pieces are utilized to quickly assemble the tetracycline core (rings A and B). Although this series of reactions is not stereoselective, the desired diastereomer is readily isolated by recrystallization or column chromatography.
Muxfeldt's synthetic route allowed for the first time the preparation and isolation of various tetracyclines with only minor changes to the original plan. In addition to the synthesis of (±)-6-demethyl-6-deoxytetracycline 42 and (±)-oxytetracycline 27, Muxfeldt and co-workers utilized their synthetic strategy for the first and only synthesis of (±)-anhydroaureomycin55 (and, by extension, Aureomycin 26, based on the procedure by Scott and co-workers).56 Moreover, their strategy was reportedly used by other workers to prepare tetracycline analogs. For example, a group at Hoechst AG prepared and studied the C-5a- and C-6-epimeric series of 7-chloro-6-deoxytetracycline in addition to C-5a-epi-7-chloro-6-demethyl-6-deoxytetracycline, as shown in structures 51 and 52.57 Tetracyclines containing a truncated B ring, the (±)-B-nortetracyclines (53), were also prepared by the Muxfeldt route.49 One of the few examples of the introduction of functionality at the C-8 position was provided in the synthesis of racemic 8-hydroxy-6-demethyl-6-deoxytetracycline 54.58 Finally, the interesting and highly active analogs (±)-6-thiatetracycline (55, X = S)59 and (±)-6-oxatetracycline (55, X = O)48 were synthesized using the Muxfeldt strategy.
There are also reported syntheses of 12a-deoxyanhydrotetracycline (56)\textsuperscript{60,61} which, when combined with work by Wasserman and co-workers describing the synthesis of 28 from anhydrotetracycline 33 (R\textsubscript{1} = H, R\textsubscript{2} = H),\textsuperscript{62} would constitute formal syntheses of tetracycline, provided that the single report of selective 12a-hydroxylation of 56 (O\textsubscript{2}, PtO\textsubscript{2}, Et\textsubscript{3}N, THF, 20 °C)\textsuperscript{63} can be verified. Similarly, 12a-deoxytetracycline (57) has been utilized as a synthetic intermediate. There are a number of methods that have been used to bring about the C-12a hydroxylation of 57 or its C-4 epimer, such as through the use of perbenzoic acid,\textsuperscript{64} sodium nitrite,\textsuperscript{65} a prehydrogenated platinum oxide catalyst with oxygen,\textsuperscript{66} or a microbiological process,\textsuperscript{67} although these generally proceed in low yield. Recently, however, Stork and co-workers reported that they were unable to reproduce the C-12a hydroxylation reactions described by Holmlund\textsuperscript{65} or Muxfeldt\textsuperscript{66} to transform 57 into tetracycline (vide infra). Similarly, the oxygenation procedure developed by Woodward (cerous chloride, oxygen, pH 10 buffer)\textsuperscript{50c} was also not successful in the Stork efforts. It should be noted that the Woodward and Muxfeldt (oxygen, triethylphosphite, sodium hydride)\textsuperscript{53} oxygenation methods were originally used to hydroxylate the C-12a position of substrates with no C-6 hydroxyl group, or with a protected C-6 hydroxyl group. Even then, the reported yields for these procedures were low (<50%), and optimization of the
reaction conditions was typically necessary for each new substrate. Thus, it is clear that the incorporation of the C-12a hydroxyl group was, and remains, the challenging problem in the synthesis of tetracycline.

Recently, Stork and co-workers reported the first stereospecific total synthesis of a tetracycline, (±)-12a-deoxytetracycline 57 (Scheme VI). Their efficient and high-yielding synthesis is outlined below. The double bond of 5-hydroxy-1,4-naphthoquinone was protected as a Diels–Alder adduct (cyclopentadiene, CHCl₃, 100%) such that only 1,2-addition to the carbonyl group could take place with methylmagnesium bromide (THF, −78→23 °C, 78%). Deprotection of the intermediate alcohol via a retro Diels–Alder reaction (p-xylene, heat, 100%) then provided the racemic alcohol 58. Reaction of 58 with the bromine adduct of ethyl vinyl ether (N,N-dimethylaniline, CH₂Cl₂, heat, 98%), and subsequent radical cyclization of the intermediate haloacetal with tributyltin hydride (AIBN, PhH, heat, 90%) afforded the cis-fused acetal 59. Protection of the masked aldehyde with 1,3-propane dithiol (BF₃•OEt₂, CH₂Cl₂, 0 °C, 88%), esterification of the tertiary alcohol with a mixed anhydride formed from allyl malonate (TFAA, DME, 92%), cleavage of the dithiane by transacetalization (PhI(OCOCF₃)₂, CH₃OH, CH₂Cl₂, 92%; 5% aq. HCl, THF), and intramolecular condensation of the malonate ester with the resultant aldehyde (piperidine, AcOH, PhH, 0→23 °C, 97%) provided the lactone 60. This tricyclic lactone (60) was then used to set the C-4a stereocenter via a stereoselective Michael addition. Deprotonation of methyl 3-benzylxyloxy-5-dimethylaminomethyl-4-isoxazolecarboxylate with sodium bis(trimethylsilyl)amide and reaction of the anion with lactone 60 (THF, −78→50 °C) gave an intermediate addition product which then underwent removal of the allyl ester group (Pd(PPh₃)₄, PPh₃, ethylhexanoic acid, EtOAc, CH₂Cl₂, 95%) to provide intermediate 61. Cleavage of the lactone within 61 with tributyltin methoxide (PhCH₃, 60 °C) afforded the cyclization precursor 62 in 97% yield. Based on their previous difficulties with forming the A ring last, it was crucial to the success of the synthesis that cyclization to form ring A occur prior to that of ring B. Thus,
Scheme VI

Reagents and conditions: (a) cyclopentadiene, CHCl₃, 100%; (b) CH₃MgBr, THF, -78→23 °C, 78%; (c) p-xylene, heat, 100%; (d) EtOCHBrCH₂Br, N,N-dimethylaniline, CH₂Cl₂, heat, 98%; (e) nBu₃SnH, AIBN, PhH, heat, 90%; (f) 1,3-propanedithiol, BF₃·OEt₂, CH₂Cl₂, 0 °C, 88%; (g) (HO₂CCH₂CO₂CH₂CH=CH₂, TFAA), DME, 92%; (h) PhI(OCOCF₃)₂, CH₂Cl₂, CH₃OH, 92%; (i) 5% HCl, THF; (j) piperidine, AcOH, 4Å molecular sieves, PhH, 0→23 °C, 97%; (k) (NaHMDS, methyl 3-benzylxoy-5-dimethylaminomethyl-4-isoxazolocarboxylate), THF, -78→-50 °C; (l) Pd(PPh₃)₄, PPh₃, ethylhexanoic acid, 4Å molecular sieves, EtOAc, CH₂Cl₂, 95%; (m) Bu₃SnOCH₃, PhCH₂, 60 °C, 97%; (n) TMSCN, KCN, 18-crown-6, CH₂Cl₂; (o) KH, THF, -78→0 °C; 23→50 °C, 59%; (p) H₂, Pd black, THF, CH₃OH, 94%.
the ketone in the C ring of 62 was protected as the trimethylsilyloxy ketal (TMSCN, KCN, 18-crown-6, CH₂Cl₂), and the resultant intermediate was treated with base (KH, THF, -78→0 °C) to effect closure of the A ring, presumably via the intermediate 63. The reaction mixture was then warmed to 50 °C to effect the second ring cyclization upon in situ cleavage of the trimethylsilyloxy ketal of ring C to provide the tetracyclic product 64 (59%). Hydrogenolysis of the isoxazole (Pd black, H₂, THF, CH₃OH, 94%) then gave the target substrate 57 in an impressive 25% yield over the 16 linear steps.

Several features of the Stork synthesis are noteworthy. The fact that this synthesis is highly stereoselective greatly simplifies product purification. All subsequent stereochemistry was dependent on the initial stereocenter within the early intermediate 58. Thus, resolution of this material would permit the isolation of 57 in optically-active form. Another significant highlight is the clever way in which the sensitive functionality of the A ring is protected. The isoxazole ring system is stable to the various reaction conditions and is unmasked at the appropriate juncture to form the desired β-keto amide through a simple hydrogenation reaction. The transformation of 57 to tetracycline by hydroxylation of the C-12a position should have been viable using one of the many published hydroxylation methods described above. Unfortunately, Stork and co-workers have been unable to duplicate any of the C-12a hydroxylation methods for the purpose of synthesizing tetracycline. To date, this C-12a hydroxylation reaction remains the key unsolved problem in the synthesis of fully functionalized tetracycline.

**Retrosynthetic Plan**

Structure-activity relationship studies have determined that substitution along the C and D rings of tetracycline produces novel, active antibiotic substances, while substitution along the A and B rings produces primarily inactive compounds. The goal of our research program was to provide a concise, enantioselective synthesis of the tetracyclines that would not only provide the first total synthesis of tetracycline but would also allow for
the preparation of a wide variety of analogs to aid in the discovery of new compounds with potentially improved pharmacological profiles.

In studies leading to the total synthesis of (+)-dynemicin A, Myers and co-workers assembled the tricyclic anthraquinone core of the molecule by an isobenzofuran Diels–Alder cycloaddition, an approach originally used by Kende and co-workers in synthetic studies toward the anthracycline antibiotics. The isobenzofuran diene components utilized in the dynemicin studies were synthesized by two different methods, the first method making use of 1,1-diehtoxyphthalan 65 as an isobenzofuran precursor. Heating a solution of 65 (5 equiv) and quinone imine 67 in toluene containing acetic acid (0.2 equiv) at reflux afforded the Diels–Alder adducts 68 and 69 in a combined yield of 56%. Adducts 68 and 69 were formed as a 1:1 mixture of endo and exo Diels–Alder products where both compounds were believed to arise from diene attack on the less hindered α-face of the dienophile.

![Diagram of Diels–Alder adducts](image)

For the synthesis of the more highly oxygenated anthraquinones in the dynemicin system, a second method of isobenzofuran generation involving a deprotonation-silylation
sequence was utilized. Treatment of the phthalide 70 with lithium bis(trimethylsilyl)amide (THF, −78 °C) and subsequent trapping of the anion with chlorotrimethylsilane presumably formed the O-trimethylsilyloxy isobenzofuran 71. Further reaction with the quinone imine 67 at ca. 65 °C provided exclusively the exo Diels–Alder adduct 72 in 34% yield. The additional steric hindrance of the isobenzofuran 71 was believed to prevent an endo-selective Diels–Alder cycloaddition. Cleavage of the trimethylsilyloxy ketal of 72 with triethylamine trihydrofluoride (CH₃CN, 23 °C) then afforded the naphthalenol 73 in 44% yield.

The application of the isobenzofuran Diels–Alder strategy to the synthesis of tetracycline would seem an almost ideal match based on the functionality present within the left-hand portion of the natural product. The use of this cycloaddition to assemble the two halves of tetracycline would provide a highly convergent and stereoselective path to the target, readily providing the β-diketone functionality of the BCD ring chromophore and potentially establishing two of five stereogenic centers in one reaction (Scheme VII, P = protecting group). The intermediate 74, for example, contains the full retron for an
isobenzofuran Diels–Alder transform leading to its assembly from the phthalide 75 and the enone 76. The synthesis of the isobenzofuran precursor (75) would follow established methodology for the synthesis of phthalides and should readily accommodate a wide range of functionality on the aromatic ring as desired for new tetracycline antibiotic candidates. The synthesis of the right-hand half (76) is much less straightforward and is the most difficult part of this retrosynthetic scheme. Intermediate 76 comprises three stereogenic centers and much of the polar and reactive functionality of the parent tetracycline. Structure-activity relationships, discussed above, suggest that the right-hand precursor of tetracycline would not require the flexibility for analog preparation that is desirable within the left-hand precursor.

Scheme VII

The feasibility of this retrosynthetic plan, however, depends upon one very important question: can the isobenzofuran Diels–Alder reaction be used to assemble the two halves of tetracycline with the requisite stereochemistry? To be useful, the isobenzofuran Diels–Alder cycloaddition must occur with exo and dienophile-facial
selectivity. As observed in the dynemicin synthetic studies discussed above, the stereochemistry of the isobenzofuran Diels–Alder reaction is very sensitive to changes in the substrate structures.\(^7\) The use of a 4,7-disubstituted isobenzofuran in conjunction with a bulky tricyclic dienophile appears to favor an exo-selective Diels–Alder reaction, a conclusion further supported by the work of Rodrigo and co-workers in which the exo Diels–Alder adduct 79 was formed exclusively from the proposed isobenzofuran 77 and the enone 78 (eq 2).\(^7\) However, as stated previously, this conclusion must await experimental proof in studies with the actual right-hand half of tetracycline. Minor changes in either substrate should prove beneficial towards tuning the Diels–Alder reaction to the desired outcome. In terms of the facial selectivity of the Diels–Alder reaction, it is hoped that a bulky protecting group for the \(\alpha\)-hydroxyl group within dienophile 76 can be used to block attack of the isobenzofuran from the \(\alpha\)-face of the dienophile. Chelation of a Lewis acid to the 1,3-dicarbonyl group of dienophile 76 may provide an additional site for controlling the selectivity of or enhancing the reactivity of the Diels–Alder reaction.

\[
\begin{align*}
\text{H}_3\text{CO} & \quad [\begin{array}{c}
\text{H}_3\text{CO} \\
\text{H}_3\text{CO}
\end{array}] \\
\text{H}_3\text{C} & \quad \text{H}_3\text{C} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
77 & \quad + \quad 78 & \quad \xrightarrow{[\text{H}^+] \Delta} \quad 79 & \quad 60\%
\end{align*}
\]

Model Studies Relevant to the Isobenzofuran Diels–Alder Reaction

Although the issue of stereochemistry in the isobenzofuran Diels–Alder reaction was postponed pending completion of a fully functionalized right-hand half, the inherent reactivity of the proposed dienophile 76 was examined briefly in model studies. Retrosynthetic analysis had provided an enone as the right-hand target, a less electron-deficient dienophile than the benzyynes, benzoquinones, and quinone imines commonly utilized in this reaction. In fact, the example shown in eq 2 is the only published example
of an isobenzofuran Diels–Alder reaction with an enone as dienophile. This reaction is not directly applicable to the proposed system, however, because it takes place under conditions that are not compatible with 1,3-disubstituted isobenzofurans, which are unstable at room temperature. Accordingly, the goal became to demonstrate that an O-trimethylsilyloxy isobenzofuran would react with an enone as dienophile under conditions compatible with the unstable isobenzofuran.

The synthesis of the isobenzofuran precursor for the left-hand half of tetracycline proceeded readily following the work of Snieckus and co-workers (Scheme VIII). Treatment of aldehyde 80, prepared from o-anisic acid, with methylmagnesium bromide (2.1 equiv) in tetrahydrofuran at 0 °C followed by cyclization of the secondary alcohol with p-toluenesulfonic acid monohydrate (1.1 equiv) in hot toluene (100 °C) afforded phthalide 81 in 89% yield. Demethylation of phthalide 81 with boron tribromide (2.4 equiv) in dichloromethane (−78→23 °C) produced 7-hydroxy-3-methylphthalide 82 in 88% yield. Protection of 82 as its tert-butyldimethylsilyl ether using TBSCI (3.0 equiv), triethylamine (4.0 equiv), and DMAP (0.1 equiv) in THF gave phthalide 83 (81%).

Scheme VIII
To accommodate functionalization on the phthalide, generation of the isobenzofuran through a deprotonation-silylation sequence was planned. The proposed isobenzofuran intermediates 84 and 85 were prepared in all cases by deprotonation of phthalides 81 or 83, respectively, with potassium bis(trimethylsilyl)amide (1.1 equiv) in THF (−78 °C, 30 min) and silylation with a mixture of chlorotrimethylsilane and triethylamine (1:1, ~3.0 equiv TMSCl; −20 °C for 0.5–3 min, then −78 °C). Immediate reaction of the unstable isobenzofuran with the dienophile at 50–60 °C then produced the Diels–Alder adducts. Condensation of isobenzofuran 84 (1.1 equiv) with p-benzoquinone provided the endo adduct 86 in ~75% yield (determined by 1H NMR analysis of the crude reaction mixture using an internal standard; eq 3). Reaction of isobenzofuran 85 with quinone ketal 87 (1.2 equiv) gave the endo Diels–Alder adducts 88 and 89 as a 1:1 mixture of diastereomers in 39% yield (56% based on recovered quinone ketal 87; eq 4). Condensation of enedione 90 with isobenzofuran 85 (1.5 equiv) provided the endo adduct 91 (60%; eq 5). Desilylation and acetal cleavage of 91 with HF•pyridine complex buffered with pyridine in THF produced the tetracyclic compound 92 in 60% yield, notably, without epimerization.
or aromatization (eq 6). Determination of the Diels–Alder selectivity for these products was based on long-range $^1H$ NMR difference NOE enhancements.

![Chemical structures](image)

To probe the use of a simple enone as a dienophile the isobenzofuran 84 (1.1 equiv) was heated with 2-cyclohexen-1-one. No adduct was observed. Similarly, reaction of isobenzofuran 85 (2.5 equiv) with enone 93 (prepared by the thermal Diels–Alder reaction of quinone ketal 87 with 2,3-dimethyl-1,3-butadiene) under the above conditions did not produce any Diels–Alder adduct.

![Chemical structures](image)

To probe systems more relevant to the synthetic plan, enone 96 was studied as a model for the B-ring of tetracycline. This compound was designed to contain α-oxygenation as well as a protected α-hydroxymethyl group that could serve as a handle for further functionalization or could, upon deprotection, activate the enone by hydrogen-bonding. Enone 96 was synthesized as outlined in Scheme IX. Sharpless asymmetric epoxidation of 2-(hydroxymethyl)-2-cyclohexen-1-one was effected in 74% yield using
tert-butyl hydroperoxide (5.0 equiv), titanium(IV) isopropoxide (5.0 equiv), (L)-diisopropyl tartrate (6.0 equiv), and 4Å molecular sieves (30% w/w) in dichloromethane at −20 °C. The reaction proceeded in ≥94% enantiomeric excess as determined by 1H NMR analysis of the corresponding Mosher ester derivative. Protection of alcohol 94 with tert-butyldimethylsilyl chloride (2.0 equiv), triethylamine (2.5 equiv), and DMAP (0.25 equiv) in dichloromethane provided ketone 95 in 91% yield. Introduction of α,β-unsaturation into this substrate proved to be a non-trivial task. After much experimentation, the following sequence was found to be the most effective. Ketone 95 was deprotonated and trapped as its trimethylsilyl enol ether in situ (LDA (1.5 equiv), TMSCl (3.0 equiv), THF, −78 °C). Addition of phenylselenenyl chloride (1.1 equiv, CH₂Cl₂, 0 °C) followed by oxidation of the resultant adduct with hydrogen peroxide (2.1 equiv, acetic acid (0.2 equiv), THF, 0→23 °C) provided the desired enone 96 in 72% overall yield.

The isobenzofuran Diels–Alder reaction was then examined with enone 96. Surprisingly, the reaction of the proposed isobenzofuran 84 (5.2 equiv) with enone 96 gave a single Diels–Alder adduct in 75% yield (higher yields were realized when the ratio
of chlorotrimethylsilane to triethylamine was <1:1). Careful analysis of the product indicated that opening of the epoxide had taken place prior to the Diels–Alder reaction to provide the adduct 98 with complete endo and facial selectivity (as determined by nOe studies). Presumably, epoxide opening occurs in situ upon heating in the presence of triethylamine. Since the isobenzofuran 84 is the more reactive diene, it reacts preferentially with the γ,δ-double bond, a phenomenon observed in other cases when 2,4-cyclohexadienones react as dienophiles. The cycloaddition takes place on the same face of the dienophile as the free hydroxyl group to generate the adduct 98 in high yield and with high selectivity.

![Chemical structures](image)

It was believed that an enone containing a 1,3-dicarbonyl unit, as in the actual right-hand half of tetracycline, would benefit from the potential for metal chelation as a means of activation of the dienophile. Thus, to more closely model the proposed right-hand portion of tetracycline, enone 100, containing an α-ester as well as a protected α-hydroxyl group, was prepared. Toward this end, ethyl 2-cyclohexanonecarboxylate was deprotonated with sodium hydride (1.1 equiv) in THF and the resulting anionic species was hydroxylated with a solution of dimethyldioxirane (1.6 equiv) in an acetone and dichloromethane mixture (−78 °C) in 89% yield. Alcohol 99 then underwent bis-silylation in the presence of lithium diisopropylamide (2.2 equiv, THF, −78 °C) and a solution of chlorotrimethylsilane and triethylamine (1:1, −3.0 equiv TMSCl, −78→23 °C) to form a trimethylsilyl enol ether intermediate containing the silylated alcohol. α-Alkylation of the TMS-enol ether with phenylselenenyl chloride (1.2 equiv, CH₂Cl₂, 0 °C) was followed by oxidation and
elimination of the phenylselenoxide (hydrogen peroxide (3.1 equiv), pyridine (2.0 equiv), CH₂Cl₂:H₂O, 0 °C) to provide the racemic enone 100 in 52% overall yield.⁸⁶

Treatment of enone 100 with the putative isobenzofuran 84 (1.3 equiv) at 55 °C provided a major Diels–Alder adduct 101 in 15% yield and a minor adduct 102 in 9% yield (26% and 17%, respectively, based on recovered 100). The major and minor adducts appeared to be diastereomers based upon analysis of their nearly identical ¹³C NMR spectra. A comparison of the coupling constant for the Diels–Alder ring fusion (CH–CH) protons of 101 and 102 (J = 7.3–8.4 Hz) versus previous endo (J = 9–10 Hz) and exo (J = 7–8 Hz) Diels–Alder adducts⁷⁶ indicated that these diastereomers likely
resulted from an exo Diels–Alder cycloaddition. This unexpected result, although not confirmed by nOe spectroscopic studies, further demonstrates the idiosyncrasies of the isobenzofuran Diels–Alder reaction. The adducts 101 and 102 are suspected to differ in their facial selectivity in the Diels–Alder reaction. However, given the dissimilarity of the adducts with the actual right-hand half of tetracycline, this lack of complete facial selectivity was not of great concern. The minor diastereomer 102, tentatively assigned as the adduct resulting from attack of the isobenzofuran on the β-face of the enone, was contaminated by two very minor products that are likely the endo Diels–Alder adducts, indicating that the reaction is not completely selective for the exo Diels–Alder addition. Nevertheless, the formation of the exo Diels–Alder adducts 101 and 102 in a combined yield of 24% by a simple thermal reaction provided support for the proposed synthetic plan.

As an alternative to the isobenzofuran Diels–Alder reaction, a Michael addition and subsequent Dieckmann condensation sequence could be utilized to join the two proposed halves of tetracycline. This approach was briefly probed with enone 96. Phthalide 81 (5.0 equiv) was deprotonated with KHMDS (5.2 equiv, THF, −78 °C) and the resulting enolate was condensed with enone 96 (−78 → 23 °C). The tricyclic product 103 was isolated in 38% yield. A minor amount of a compound tentatively identified as the simple Michael addition product was also isolated (19%). Analysis of 103 by nOe experiments indicated the presence of one inverted stereogenic center (C-6 position) relative to tetracycline. The decreased control over stereochemistry makes this route less attractive for the total synthesis of tetracycline.
Studies Toward the Synthesis of the Right-Hand Half of Tetracycline

Encouraged by the favorable results of the isobenzofuran Diels–Alder reactions with various dienophiles, especially the β-keto ester 100, the goal became the preparation of the fully functionalized right-hand half of tetracycline, ideally a protected form of the enone 104. It was envisioned that the use of a bulky protecting group, such as a tert-butylidemethylsilyl ether, on the tertiary alcohol would influence the facial selectivity of the isobenzofuran Diels–Alder reaction. It was also desirable to find a practical protection scheme for the polar and acidic functionality of the β-keto amide. A dioxinone framework, containing a protected β-keto ester, appeared ideal for the job at hand. These substrates are readily converted into β-keto amides upon mild heating in the presence of an amine (such as ammonia). Thus, enone 105 was selected as a suitably-protected precursor for the right-hand half of tetracycline.

Retrosynthetic analysis of enone 105 suggested the cleavage of the A ring as shown to preserve the dioxinone framework (Scheme X). Retrosynthetic cleavage of the top bond was performed first since it was thought that the C-4a stereocenter could be controlled better via an intramolecular reaction. An extended Michael-type reaction of the rigid dienolate generated within 106 onto the 2,4-cyclohexadienone subunit (a 6-exo-trig reaction) was anticipated to correctly set the C-4 and C-4a stereocenters (although correct introduction of the C-4 stereocenter would not be crucial, considering that it could be equilibrated to the desired epimer at the final stage of the synthesis). Based on previous findings from the isobenzofuran Diels–Alder reaction with enone 96, this unstable
intermediate was envisioned to arise from the in situ base rearrangement of epoxide 107. No literature precedent existed for a direct intramolecular nucleophilic addition of an enolate on to the epoxide of 107. The epoxide 107 could be prepared by the oxidation of alcohol 108, which would arise from an aldol reaction between aldehyde 109 and the functionalized dioxinone core 110. Efforts directed toward the preparation of 105 are now described.

**Scheme X**

Dimethylamine 110 was readily prepared from the product of chlorination of commercially available 2,2,6-trimethyl-4H-1,3-dioxin-4-one (Scheme XI). Efforts to displace 6-chloromethyl-2,2-dimethyl-1,3-dioxin-4-one directly using dimethylamine as a nucleophile were unsuccessful; however, reaction with sodium azide (1.1 equiv) in N,N-dimethylformamide resulted in clean conversion to the azide 111 (94%). Reduction of the azide via the Staudinger reaction (triphenylphosphine (1.1 equiv), water (1.5 equiv), THF) provided the primary amine 112 in 92% yield. Amine 112 then underwent reductive alkylation with formaldehyde (3.0 equiv) and sodium triacetoxyborohydride (4.0
equiv) in dichloroethane (0.15 M) to afford the desired dimethylamine 110 as a pale yellow solid (76%).

**Scheme XI**

\[ \begin{align*}
\text{Cl} & \text{NaN}_3 \xrightarrow{\text{DMF, 23 °C}} \text{N}_3 \\
\text{O} & \text{PPh}_3, \text{H}_2 \text{O} \xrightarrow{\text{THF, 23 °C}} \\
\end{align*} \]

The dienolate of the parent structure, 2,2,6-trimethyl-4H-1,3-dioxin-4-one, tends to favor addition to aldehydes at its γ-carbon.\(^9\)\(^9\) It was unclear what influence the dimethylamino group would have on the reactivity of the dienolate of 110, and therefore a few control reactions were performed. Amine 110 (2.1 equiv) was deprotonated with lithium diisopropylamide (2.2 equiv) in THF (−78 °C, 35 min) and the resulting dienolate was condensed with benzaldehyde to give the α-adduct 113 in 72% yield. The regiochemistry of the addition was determined by an NMR attached proton test (APT) experiment. Interestingly, when 110 (1.2 equiv) was deprotonated with LDA (1.3 equiv) in THF (−78 °C) and the resulting dienolate was treated with benzoyl cyanide, only the γ-addition product 114 was observed (19%). When KHMDS was utilized to deprotonate
amine 110 (THF, −78 °C), γ-addition products were observed exclusively (vide infra). Thus, it appeared that dimethylamine 110 could be utilized for nucleophilic addition through either the α- or γ-position of its dienolate by simply changing the electrophile or the counterion of the dienolate.

Having demonstrated the desired α-reactivity of amine 110 in an aldol reaction, efforts were then directed toward preparation of the B-ring precursor. The synthesis of aldehyde 109 proceeded from the protected enone 96, which was desilylated with trifluoroacetic acid (2.5 equiv) in a mixture of tetrahydrofuran and water (1:1) at 0 °C to afford the volatile alcohol 115 (79%). Subsequent oxidation of the alcohol to the aldehyde proceeded only with Dess–Martin periodinane (1.2 equiv) in dichloromethane.96 The isolation of 109 was complicated by its instability toward both aqueous work-up and silica gel; by-products were largely removed by multiple filtrations of the reaction mixture through Celite. The crude aldehyde 109 (excess) was then added to a solution of the lithium dienolate of amine 110 (generated with LDA in THF at −78 °C). Unfortunately, this reaction merely led to the rapid decomposition of aldehyde 109.

In an effort to increase the stability of aldehyde 109, the carbonyl group was replaced with a methyloxime group. A more rapid and efficient synthesis of the target substrate was realized when the methyloxime functionality was introduced prior to double bond formation (Scheme XII). Thus, alcohol 94 was treated with methoxylamine
hydrochloride (2.0 equiv) and pyridine (3.0 equiv) in methanol to provide methyloxime 116 in 84% yield. Deprotonation of methyloxime 116 with n-butyllithium (2.2 equiv, THF, –78 °C) and subsequent reaction with phenylselenenyl chloride (1.2 equiv, –78 °C) afforded the α-selenenylation product. This selenide was subjected in crude form to oxidation conditions (m-CPBA (1.5 equiv), CH$_2$Cl$_2$, –78→0 °C) which, upon elimination of the intermediate selenoxide, produced the α,β-unsaturated methyloxime 117 in 59% overall yield (12% recovered 116). It was gratifying to observe that oxidation of 117 with Dess–Martin periodinane (1.5 equiv, CH$_2$Cl$_2$) afforded a product that was stable to the standard reductive aqueous work-up. In addition, aldehyde 118 could be quickly purified by flash column chromatography, although it was typically used in crude form (~89%).

Aldol reactions between the lithium dienolate of amine 110 and aldehydes 118 and 119 (prepared by the oxidation of 116 with Dess–Martin periodinane) were carried out as described above. An aldol reaction with aldehyde 119 gave the α-adducts 120 and 121 as separable diastereomers (18% and 7%, respectively, 46% combined yield based on recovered 119; eq 7). The stereochemical assignment of the diastereomers was chosen arbitrarily. Similarly, an aldol reaction with aldehyde 118 produced α-adduct 122 (7%,
significant amounts of starting material remained), based on its similarity to adduct 120 (eq 8). The diastereomer of 122 was visible by TLC; however, this more polar product could not be isolated chromatographically. The yields of these reactions were not significantly improved by the use of a titanium dienolate (generated from the lithium dienolate of amine 110 and chlorotitanium triisopropoxide) or with additives (such as CeCl₃), although the reaction was selective for one diastereomer when using the titanium dienolate. When the potassium dienolate of amine 110 (1.5 equiv; generated with KHMDS (1.7 equiv) in THF, −78 °C) was condensed with aldehyde 118, only the γ-addition product 123 was observed (>18% yield; eq 9).
The inability to produce significant amounts of the desired aldol adduct led to the exploration of the use of an activated ester as the electrophile. Oxidation of aldehyde 118 with sodium chlorite (10.0 equiv) in the presence of sodium dihydrogenphosphate (13.4 equiv) and 2-methyl-2-butene (28.3 equiv) in a mixture of tetrahydrofuran and water provided carboxylic acid 124 in 73% yield (Scheme XIII). Acid chloride 125 was then prepared from 124 in situ (oxalyl chloride (5.0 equiv), DMF (cat.), CH₂Cl₂). Similarly, activated ester 126 was prepared in situ by treatment of carboxylic acid 124 with 1,1'-carbonyldiimidazole (1.1 equiv) in THF (23 °C, 1.5 h). Attempts to couple the dienolate of amine 110 (formed with either LDA, KHMD, or LHMDS) with acid chloride 125 and imidazolide 126 were surprisingly unsuccessful. Either no reaction was observed or amide formation with the amide base occurred.

Scheme XIII

![Scheme XIII](image_url)
It still remains unclear as to why the methyloxime series of substrates did not perform better in the above reactions. This may be a reflection of the steric hindrance imparted by the quaternary α-center of the aldehydes 118 and 119 and activated esters 125 and 126. The proposed order of steps in the synthesis of the right-hand half was therefore reversed (Scheme XIV). Thus, the later formation of the lower ring bond was expected to proceed more favorably by an intramolecular reaction with an aldehyde (128, \( X = H \)) or an activated ester (128, \( X = Cl \), imidazole). The formation of substrate 128 by the intermolecular addition of the potassium dienolate of amine 110 to pre-formed 2,4-cyclohexadienone substrate 129 via an extended Michael reaction was then targeted.

**Scheme XIV**

The diene methyloxime 131 was prepared as follows. Aldehyde 118 was oxidized to the corresponding carboxylic acid as described above, and, after extractive isolation, the crude acid was treated with (trimethylsilyl)diazomethane (1.8 equiv) in a solution of methanol in benzene to provide methyl ester 130 in 70% yield from 118.\(^{98}\) Reaction of ester 130 with excess triethylamine (10.0 equiv) in refluxing dichloromethane gave the...
intermediate dienyl alcohol which was protected in situ as its trimethylsilyl ether 131 by addition of trimethylsilyl trifluoromethanesulfonate (2.0 equiv) at 0 °C (85% overall yield).

With the conjugated methyloxime 131 in hand, efforts to couple it with amine 110 via an extended Michael reaction were initiated. The potassium dienolate of dimethylamine 110 was initially prepared to favor γ-addition (1.0 equiv KHMDS, THF, −78 °C) and was added in excess to a solution of methyloxime 131 in tetrahydrofuran. Minor amounts of desilylated starting material, but no adduct formation, were observed. Addition of 131 to the lithium dienolate of dimethylamine 110 was also unsuccessful. The trimethylsilyl enol ether of dimethylamine 110 was prepared (KHMDS (1.1 equiv), THF, −78 °C; chlorotrimethylsilane (2.0 equiv), −78→23 °C; characterized by 1H NMR) and combined in excess with methyloxime 131. Analysis of the crude reaction mixtures indicated that decomposition of the trimethylsilyl enol ether had occurred. The addition of Lewis acids to
these reactions gave no improvement. In all cases, the conjugated methyloxime 131 remained unchanged.

Although substitution of the carbonyl group with a methyloxime group provided enhanced stability, these compounds were ultimately too unreactive for the purposes described here. Conversion of these advanced intermediates to the corresponding ketones was then pursued, but cleavage of the methyloxime functionality at various stages (substrates 130 and 131) was unsuccessful.99–101

Because the oxidation of alcohol 115 to the aldehyde was problematic, an alternative route to the more advanced ester precursor was sought. 2-Carbethoxy-2-cyclohexen-1-one was readily prepared from 2-carbethoxycyclohexanone by the method of Reich and co-workers.86 Treatment of the unstable crude enone with tert-butyl hydroperoxide (3.0 equiv) and sodium hydroxide (0.1 equiv) in ice-cooled ethanol provided the stable, racemic epoxide 132 in 65% yield.102 In situ deprotonation and silylation of ketone 132 (LDA (1.2 equiv), TMSCl (2.5 equiv), THF, −78 °C) followed by the addition of phenylselenenyl chloride (1.2 equiv) in dichloromethane (0 °C) gave an α-phenylselenide. Direct oxidation of the crude selenide and subsequent selenoxide elimination then ensued upon treatment with hydrogen peroxide (2.2 equiv) in a mixture of dichloromethane and water (0→23 °C) to provide the enone 133 in 29% yield from ketone 132.

Transformation of the enone 133 into the corresponding 2,4-cyclohexadienone and its subsequent reaction in situ with the dienolate of amine 110 via an extended Michael addition was then pursued. Enone 133 was combined with trimethylsilyl enol ether 134
(6.9 equiv) in THF (−78→23 °C) in the presence of triethylamine (6.9 equiv). Lactone 135, characterized by 1H NMR, 1H-1H decoupling, 13C NMR, and FTIR analysis, was the only product observed (12% isolated yield). The treatment of enone 133 alone with triethylamine (5.0–10.0 equiv) in THF (0.035–0.047 M) resulted in the formation of the lactone 135 (19–34%) as well as the dimer 136 (13–19%). The latter product is believed to arise by an inverse electron demand Diels–Alder reaction of the intermediate 2,4-cyclohexadienone, which acts as both the diene and the dienophile. According to literature precedent, the reaction is believed to have proceeded with endo selectivity and the designated regiochemistry.87 The (racemic) product 136 appeared by 1H NMR to be a single diastereomer, tentatively assigned as the one in which the hydroxyl groups are in close proximity in the transition state of the cycloaddition reaction.87d

\[
\begin{align*}
\text{CH}_3 & \quad \text{O} \quad \text{O} \\
\text{H}_3\text{C} & \quad \text{N} \quad \text{TMS} \\
\end{align*}
\]

To avoid the dual reactivity displayed by an enone containing an α-ester moiety, dienone formation from enone 96 was investigated. Reaction of the optically pure enone 96 with trimethylsilyl enol ether 134 (3.4–4.8 equiv) in the presence of triethylamine (10.0 equiv) or a mixture of chlorotrimethylsilane and triethylamine (1:1, ~10.0 equiv Et₃N) in THF (0.032–0.039 M) from −78→50 °C did not lead to the desired adduct
formation. Instead, the Diels–Alder dimer 137 was isolated as a single diastereomer in 35–40% yield. Again, the facial selectivity of the Diels–Alder reaction was presumed to occur through a transition state placing the hydroxyl groups in close proximity.

The above results suggested that the desired 2,4-cyclohexadienone intermediate was not isolable in monomeric form. In the case of enone 133, the intermediate dienone underwent ring expansion, presumably favored by the presence of the electron-withdrawing ester moiety (Figure 8). In contrast, enone 96, without the electron-withdrawing group, cannot undergo this ring-expansion reaction. Thus, enones such as

![Proposed mechanism for dienone formation and rearrangement.](image)

**Figure 8.** Proposed mechanism for dienone formation and rearrangement.
were shown to be ineffective substrates for a route utilizing an intermediate 2,4-cyclohexadienone. These results also suggested that the trimethylsilyl enol ether 134 was not reactive enough for use in an extended Michael addition reaction with the dienone.

The next step was to discern whether these 2,4-cyclohexadienone intermediates could be formed under the influence of a strong base at lower temperatures such that the more reactive dienolate of amine 110 could be used as the nucleophilic species. To test this, a tetrahydrofuran solution of enone 96 was added to an excess of the potassium dienolate of dimethylamine 110 (2.3 equiv) in THF and the resultant reaction mixture was then warmed from $-78 \rightarrow 10 \, ^\circ\text{C}$. The enone was converted to phenol 138 (44%) at low temperature (eq 10). Treatment of enone 96 with LHMDS (1.1 equiv) in tetrahydrofuran ($-78 \, ^\circ\text{C}$) and subsequent reaction of this solution with the potassium dienolate of amine 110 (1.2 equiv) in THF ($-78 \rightarrow 10 \, ^\circ\text{C}$) provided phenol 138 (1%) and catechol 139 (20–30%; eq 11). These results indicated that enone 96 was undergoing deprotonation and subsequent ring opening of the epoxide, but that the dienone alkoxide intermediate was apparently rearranging rapidly to the aromatic products. In an attempt to access the intermediate dienone alcohol rather than the dienone alkoxide, a catalytic base deprotonation
system (amine 110 (1.1 equiv), KOt-Bu (1.3 equiv), DMSO, THF, −20→−10 °C) was examined, but phenol 138 was still the major product (29%).

From these results, it seemed likely that trapping the dienone alkoxide intermediate as an ether might prevent the substrate from undergoing rearrangement to the aromatic products, and the formation of a 2,4-cyclohexadienone silyl ether in situ was next examined. It was found that when enone 96 was deprotonated with KHMDS or LHMDS in the presence of chlorotrimethylsilane and then condensed with the potassium dienolate of amine 110, the dimer product 140 was observed as a single (enantiomerically pure) diastereomer. This time, it was expected that the bulky trimethylsilyl ether groups were on the outer face of the transition state, leading to the diastereomer shown. This result provided evidence that the silylated 2,4-cyclohexadienone 141 was in fact being generated.

Formation of the silylated dienone 141 (140 (1.0 equiv), LHMDS (1.2 equiv), TMSCl (1.3 equiv), THF, −78 °C) under more dilute conditions (0.04 M vs 0.15 M) and subsequent transfer of the potassium dienolate of dimethylamine 110 (2.8 equiv) via cannula into the unstable dienone solution (−78→−15 °C) did, in fact, provide an adduct (142, 13% yield) in addition to the phenol 138 (30%) and the diol 139 (8%). However,
product 142, isolated as a 1:2.5 mixture of diastereomers, presumably both from attack at the β-face of the dienone, was determined to be the 1,4-Michael addition product by $^{13}$C NMR analysis.

**A Route to the Right-Hand Half by Phenolic Oxidation**

Further examination of the literature of 2,4-cyclohexadienone substrates provided useful information concerning dienone synthesis and stability. 6-Hydroxy-6-alkyl-2,4-cyclohexadienones such as 143 can be obtained in situ through the retro Diels–Alder reaction of the dimer 144. The monomers dimerize at ambient temperature or above, depending upon the stereoelectronics of the substrate. The initial preparation of the cyclohexadienone, and subsequently its dimer, proceeds via the ortho oxidation of a phenol precursor with, for example, an oxidant such as sodium periodate. Alternatively, dimer 144 can be accessed through the hydrolysis of a 6-acetoxy-6-alkyl-2,4-cyclohexadienone such as 145. The presence of a 6-acetoxy group apparently makes the monomer dienone substrate more stable, even allowing for its purification by column chromatography. Compounds such as 145 are typically synthesized by the Wessely oxidation in one step from the substituted phenols. This transformation, which utilizes lead(IV) acetate as the oxidant typically in acetic acid as the solvent, provides products with $\alpha$-oxidation preferentially at the substituted carbon and operates optimally with electron-rich aromatic rings.
The direct oxidation of a phenolic system to access a 2,4-cyclohexadienone intermediate would provide a much more concise route to the synthesis of the right half of tetracycline since the starting materials would be commercially available or trivial to prepare. Interestingly, Barton and co-workers researched this strategy for the synthesis of the A ring of tetracycline 28 (Scheme XV).\textsuperscript{107} Under their plan, the crucial oxygenation at C-12a would arise from the dearomatization of the A ring via phenol oxidation of intermediate 148. Although several conditions were investigated for this transformation, Barton and co-workers were only able to \(\alpha\)-hydroxylate simple one-ring aromatic phenols.

\textbf{Scheme XV}

\[\text{Scheme XV}\]

\[
\begin{align*}
\text{28} & \quad \Longrightarrow \quad \text{146} \\
\text{147} & \quad \Longrightarrow \quad \text{148}
\end{align*}
\]

For this work, the suggested stability of the 6-acetoxy-2,4-cyclohexadienones warranted an evaluation of their use in the synthetic plan for the right half of tetracycline. The most obvious way to incorporate this change would be to prepare the cyclohexadienone 150 from Wessely oxidation of the phenol precursor 149 as a stable (racemic) starting material for more controlled examination of an extended Michael addition reaction with the dienolate of amine 110. Alternatively, the stable cyclohexadienone 150
could serve as a new starting material for subsequent aldehyde formation to give the proposed substrate 152. An aldol reaction between 152 and the lithium dienolate of dimethylamine 110 could potentially provide adduct 153, which could then undergo a regiochemically and stereochemically more favorable intramolecular addition to the conjugated dienone.

To test the feasibility of the Wessely oxidation for the one step preparation of 2,4-cyclohexadienones from simple phenol precursors, 2-hydroxybenzyl alcohol was protected as its silyl ether with the more acid stable triisopropylsilyl group. Thus, triisopropylsilyl ether 154 was prepared by treatment of the alcohol with TIPSOTf (1.1 equiv) and triethylamine (1.5 equiv) in dichloromethane (0 °C) in 92% yield. Oxidation of phenol 154 with lead(IV) acetate (1.5 equiv) in acetic acid did provide the 2,4-cyclohexadienone 155 as a stable yellow oil in 27% yield.106,108 None of the desired product was observed upon
phenol oxidation of 154 with iodobenzene diacetate, iodobenzene bis(trifluoroacetate), or manganese(III) acetate.\textsuperscript{109,110} The further functionalization of cyclohexadienone 155 was not pursued.

Perhaps a more exciting way to incorporate this methodology for the formation of a stable 2,4-cyclohexadienone would be to oxidize a highly functionalized phenol precursor, such as substrate 156. The resultant 2,4-cyclohexadienone 157 would then need only undergo intramolecular addition, perhaps even under the reaction conditions for the oxidation, to effect the formation of the AB ring system (158). Such a route would constitute a remarkably rapid synthesis of the bicyclic right half of tetracycline.

![Chemical structures](image)

This synthetic strategy was pursued in the following manner. Salicylaldehyde was protected as its tert-butyldimethylsilyl ether 159 with TBSCl (2.0 equiv), triethylamine (3.0 equiv), and DMAP (cat.) in tetrahydrofuran in quantitative yield (Scheme XVI). An aldol reaction between the lithium dienolate of amine 110 (generated with LDA (1.2 equiv) in THF at \(-78^\circ\text{C}\)) and aldehyde 159 (1.3 equiv, THF, \(-78^\circ\text{C}\)) provided alcohol 160 in 76% yield. Oxidation of the resultant secondary alcohol to a ketone proved to be a non-trivial procedure. The use of Dess–Martin periodinane, pyridinium dichromate, pyridinium chlorochromate, or Swern conditions was unsuccessful.\textsuperscript{96,111} However, prior treatment of alcohol 160 with trifluoroacetic acid (1.1 equiv, CH\(_2\)Cl\(_2\), 0 \(^\circ\text{C}\)) to protonate the dimethylamino group and subsequent oxidation with Dess–Martin periodinane (1.5 equiv, 23 \(^\circ\text{C}\)) resulted in the formation of ketone 161 in 79% yield.
Scheme XVI

In order to test the Wessely oxidation, the deprotection of alcohol 160 was first necessary. Desilylation of 160 with triethylamine trihydrofluoride in dichloromethane gave the desired diol 162 by analysis of the crude $^1$H NMR. Although the product was visible by TLC, the isolation and further characterization of 162 proved difficult due to its conversion to the less polar compound 163 with time or under acidic conditions. This by-product is proposed to form via the dehydration of diol 162 and the subsequent electrocyclic rearrangement of the intermediate quinone methide (Figure 9). Nevertheless, oxidation reactions were carried out on the crude diol 162 before its conversion to the
rearranged amine 163. Reaction of diol 162 with lead(IV) acetate in acetic acid or dichloromethane, however, resulted only in the rapid decomposition of the crude starting material.

Figure 9. Proposed mechanism for a quinone methide rearrangement to generate 163.

Due to the lability of the benzyl alcohol of 162, a protected version of this compound was targeted. Protection of the secondary alcohol as a tert-butyldimethylsilyl or triisopropylsilyl ether proceeded readily; however, selective deprotection of the TBS aryl ether was difficult. The mono-protected phenols were unstable, and efforts to oxidize the phenols with lead(IV) acetate to the desired 2,4-cyclohexadienone substrates were unsuccessful.

An alternate way to avoid the formation of dimethylamine 163 from substrates with a labile benzylic position would be to make use of the advanced ketone substrate. Therefore, the deprotection and subsequent phenol oxidation of ketone 161 was pursued. The desilylation of ketone 161 was realized using the HF•pyridine complex buffered with pyridine in tetrahydrofuran. The keto alcohol 164 was isolated in low crude yield (<50%).
With phenol 164 in hand, efforts turned toward its oxidation to the desired 2,4-cyclohexadienone product. Oxidation of phenol 164 with lead(IV) acetate in acetic acid proved disappointing. In dioxane, a new product was observed containing altered methylene protons but no acetate group. Decomposition of the starting material was observed in other solvents, even with prior protonation of the dimethylamino group with trifluoroacetic acid. A wide range of oxidants were also examined, to include benzoyl peroxide, sodium periodate, sodium tetraperoxymolybdate, iodobenzene diacetate, iodobenzene bis(trifluoroacetate), dimethyldioxirane, and m-chloroperoxybenzoic acid, but decomposition of the starting material was observed in almost every case. Some insight into the reactions performed under acidic conditions was provided by following the reaction of phenol 164 and iodobenzene bis(trifluoroacetate) in acetic acid-$d_6$ by $^1$H NMR. It was found that phenol 164 did not react with the reagent under these conditions. Rather, slow hydrolysis of the ketal group and subsequent decomposition of the starting material was observed. Notably, the methylene protons of phenol 164 did not undergo deuterium exchange in the acidic medium as they typically did in related systems (vide infra).

The difficulties experienced in the oxidation attempts with diol 162 and phenol 164 demonstrate the weaknesses of the Wessely oxidation. Its sensitivity to the electronic nature of the aromatic ring typically results in low yields and mixtures of products. To circumvent this problem, a more electron-rich system was considered. The strategic placement of an additional hydroxyl group on the aromatic ring now allowed the option of synthesizing a 2,5-cyclohexadienone substrate (166, Scheme XVII). The $p$-oxidation of
phenols, typically performed with iodobenzene diacetate or iodobenzene bis(trifluoroacetate), is a more common and reliable transformation than the o-oxidation of phenols.\(^{114}\) Such a 2,5-cyclohexadienone 166 could then undergo an intramolecular Michael addition to form the bicyclic substrate 167. Selective reduction of the B-ring ketone of 167 followed by deprotection of the ether and elimination of the alcohol under acidic conditions would effect the transposition of the enone to give the target substrate 168.

This route was pursued in the following manner (Scheme XVIII). Commercially available 4-hydroxy-2-methoxybenzaldehyde was protected as its tert-butyldimethylsilyl ether 169 with TBScI (1.4 equiv) and triethylamine (1.5 equiv) in tetrahydrofuran (89%). Condensation of the lithium dienolate of amine 110 (generated with LDA (1.1 equiv) in THF, \(-78 \, ^\circ\text{C}\)) with aryl aldehyde 169 (1.2 equiv, THF, \(-78 \, ^\circ\text{C}\)) provided the aldol adduct 170 in 81% yield. Oxidation of alcohol 170 to ketone 171 was accomplished in 77% yield by protonation of the dimethylamino group of 171 with TFA (1.1 equiv) in dichloromethane (0 °C) and subsequent oxidation with Dess–Martin periodinane (1.5 equiv, 23 °C).
Deprotection of the adduct 170 required considerably stronger reaction conditions than in previous systems. Desilylation of 170 was executed with tetrabutylammonium fluoride (1.1 equiv) in tetrahydrofuran (0 °C) to give diol 172 in 82% yield. This compound was stable to column chromatography. In fact, most of the substrates in this series, diol 172 included, were isolated as solids.

Oxidation of diol 172 to a 2,5-cyclohexadienone substrate was briefly pursued. Treatment of diol 172 with iodobenzene bis(trifluoroacetate) (2.1 equiv) in acetic acid gave 2-methoxy-1,4-benzoquinone as the major product. This product may form via oxidative
assistance from the benzylic alcohol. When this reaction (1.1 equiv oxidant) was performed in trifluoroethanol with excess sodium acetate (10.0 equiv), a multitude of unidentifiable polar products were produced.

![Chemical structure](image)

Efforts were then focused on the more advanced intermediate, ketone 171. The desilylated ketone would appear to be an ideal starting material for phenol oxidation because of the lability of its methylene protons. In ketones 161 and 171, the methylene protons are readily exchanged in deuterated solvent. Thus, an intramolecular cyclization on to the intermediate dienone may even occur in situ. The methylene protons of ketone 161 underwent deuterium exchange with methanol-\(d_4\) (30.0 equiv) in CDCl\(_3\) (0.013 M) with a half-life of 10 h. In acetic acid-\(d_6\), however, this substrate was subject to ketal hydrolysis. Similarly, the methylene protons of ketone 171 displayed deuterium exchange with methanol-\(d_4\) (30.0 equiv) in CDCl\(_3\) (0.014 M) with a half-life of 26 h. In acetic acid-\(d_6\), this exchange was very rapid (\(t_{1/2} = <5\) min). By comparison, the direct aldol adduct 170 showed absolutely no deuterium incorporation in acetic acid-\(d_6\).

Deprotection of the TBS ether of ketone 171 was accomplished with the HF•pyridine complex buffered with pyridine in THF. Similar to the deprotection of ketone 161, phenol 173 was only isolated in low crude yield (\(\leq 50\%\)). Phenol 173 was found to
be stable in acetic acid by $^1$H NMR analysis and underwent only minimal decomposition after 4 h.

The $p$-oxidation of phenol 173 was then studied in depth using as reagents iodobenzene diacetate, iodobenzene bis(trifluoroacetate), lead(IV) acetate, and manganese(III) acetate. These oxidants were all used in excess (1.5–7 equiv). Experiments performed in solvents other than acetic acid were also performed in the presence of trifluoroacetic acid (1 equiv) to protonate the dimethylamino group prior to addition of the oxidant. The desired product was not observed in any of these reactions. 2-Methoxy-1,4-benzoquinone was produced when using iodobenzene bis(trifluoroacetate) as the oxidant. The most interesting results occurred when using iodobenzene diacetate in acetic acid and iodobenzene bis(trifluoroacetate) and dry sodium acetate (10 equiv) in trifluoroethanol. In these experiments, very polar compounds were produced that were extremely difficult to isolate due to their affinity for silica gel and their overall insolubility in most organic solvents.

![Chemical structure of 173](image)

In an effort to determine the nature of the polar products that were being formed, oxidation reactions of phenol 173 in acetic acid-$d_6$ were followed by $^1$H NMR. In the presence of iodobenzene bis(trifluoroacetate) (1.1 equiv), phenol 173 underwent complete conversion to an intermediate containing both of the starting materials. Although iodobenzene was then released, none of the desired product was observed. Instead, two singlets were observed in the aromatic region. Hydrolysis of the ketal from this intermediate was eventually observed.
There are reports in the literature concerning the oxidation of phenols with iodobenzene diacetate that contain an electron-withdrawing group in the para position. Such species were reported to favor ylide formation, as depicted in Figure 10 (EWG = electron-withdrawing group). A phenol (174) reacts with the reagent to first give complex 175, which, in aromatic rings containing an electron-withdrawing group in the para position, produces the iodonium salt 176. Ylide 176 is described in the literature as a highly polar compound that is commonly not soluble in organic solvents, similar to the observations in the above experiments. Ylide 176 can then react with nucleophiles to form a substituted phenol with the displacement of iodobenzene or rearrange with heat to form an iodoaryl phenyl ether. Based on this information, ylide formation at the least hindered α-site of 173, and perhaps subsequent nucleophilic attack by an acetate on the ylide carbon, is likely to have occurred in the above reactions.

![Proposed formation of ylides under oxidative conditions.](image)

With these results, focus returned to the substrates in which the benzylic carbon was at the alcohol level of oxidation. The use of an internal nucleophile to assist in 2,5-cyclohexadienone formation, thus giving a protected hydroxyl group at C-12a, was very appealing. This approach was first demonstrated by Tamura and co-workers to prepare spirolactones from the carboxylic acid or amide precursors under oxidative conditions. Thus, phenolic amide 177 was cyclized to spirolactone 179 in good yield upon treatment with iodobenzene bis(trifluoroacetate) in acetonitrile. With this precedent, the goal became
to determine whether oxidative spirolactonization could also occur from internal nucleophilic addition by a carbamate.

\[
\begin{align*}
&\text{HO-} \begin{array}{c} \text{NHB} \end{array} \text{N-} \begin{array}{c} \text{Bn} \end{array} \text{O} \quad \text{Ph}(\text{OCOCF}_3)_2 \quad \text{CH}_3\text{CN} \quad \text{177} \\
&\text{H}_2\text{O} \quad \text{178} \quad \text{179}
\end{align*}
\]

The synthesis of carbamate 181 was therefore pursued (Scheme XIX). Deprotonation of aldol adduct 170 with sodium hydride (5.5 equiv) in THF (0 °C) followed by acylation of the resulting alkoxide with dimethylcarbamyl chloride (10.0 equiv, 0→23 °C) provided carbamate 180 in 39% (unoptimized) yield along with 28% recovered starting material. Desilylation of the TBS aryl ether 180 with tetrabutylammonium fluoride (1.5 equiv) and acetic acid (3.5 equiv) in THF (0 °C) afforded the unstable phenol 181, which was used in crude form in subsequent reactions.

**Scheme XIX**

\[
\begin{align*}
&\text{TBSO} \quad \text{170} \\
&1. \text{NaH, THF, 0 °C} \quad \text{NaH, THF, 0 °C} \quad \text{TBSO} \\
&2. \text{dimethylcarbamyl chloride, 0→23 °C} \quad \text{170} \quad \text{39%} \quad \text{180}
\end{align*}
\]

\[
\begin{align*}
&\text{TBAF, AcOH} \quad \text{THF, 0 °C} \quad \text{170} \\
&\text{181}
\end{align*}
\]
A few preliminary attempts were made at the oxidation of phenol 181 to the desired spirocyclohexadienone. Reaction of phenol 181 with iodobenzene bis(trifluoroacetate) (2.4 equiv) in trifluoroethanol resulted in the rapid decomposition of the starting material. The use of the milder oxidant iodobenzene diacetate (1.4 equiv) in trifluoroethanol provided similar results. Finally, the reaction course of phenol 181 was observed by $^1$H NMR in acetic acid-$d_6$ (the starting material was stable in this solvent). The addition of iodobenzene diacetate (1.2 equiv) resulted in significant and immediate decomposition of the starting material. Product formation was not observed. Although these preliminary results were not encouraging, this study of the oxidation of phenol 181 is not complete, and further experimentation is warranted.

**Conclusion**

The research presented here has led to a concise strategy for the completion of the total synthesis of tetracycline. The left-hand half, phthalide 83, has been prepared in 5 steps in 56% overall yield from commercially available starting material. Several isobenzofuran Diels–Alder reactions have been carried out in an effort to model the proposed condensation of the two halves of tetracycline. Specifically, a thermal Diels–Alder reaction has been shown to be successful with an enone dienophile containing a 1,3-dicarbonyl unit ($84 + 100 \rightarrow 101 + 102$), proceeding with apparent exo selectivity. Dimethylamine 110 was prepared in 3 steps in 66% yield from 6-chloromethyl-2,2-dimethyl-1,3-dioxin-4-one as a protected, yet fully functionalized, right-hand half of the A ring of tetracycline. Several studies aimed at the annulation of amine 110 to a suitable B-
ring precursor to provide the proposed bicyclic right-hand half of tetracycline have been described. Finally, a novel and potentially rapid route to this right-hand half involving the preparation of intermediate 2,4-cyclohexadienones or 2,5-cyclohexadienones via oxidation of phenol precursors has been briefly examined. With further study, it is believed that this route will, in fact, lead to the elusive right-hand half and subsequently to completion of the total synthesis of tetracycline.
Experimental Section

**General Procedures.** All reactions were performed in flame-dried round bottom or modified Schlenk (Kjeldahl shape) flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Where necessary (so noted), reaction mixtures were degassed by alternate evacuation/argon-flush cycles (five or more iterations). Organic solutions were concentrated by rotary evaporation at \(-25\) Torr (water aspirator). Flash chromatography was performed as described by Still et al.,\(^{33}\) employing 230–400 mesh silica gel. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with 0.25 mm 230–400 mesh silica gel containing a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light and/or exposure to an acidic solution of \(p\)-anisaldehyde or ceric ammonium molybdate followed by heating on a hot plate.

**Materials.** Commercially available reagents were used as received with the following exceptions. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Benzene, toluene, dichloromethane, diisopropylamine, chlorotrimethylsilane, pyridine, and triethylamine were distilled from calcium hydride at 760 Torr. Methanol was distilled from magnesium at atmospheric pressure. Dimethylcarbamyl chloride, titanium(IV) isopropoxide, and benzaldehyde were distilled at 1 Torr. Anhydrous \(N,N\)-dimethylformamide was purchased from Aldrich Chemical Co. and was stored over 4Å molecular sieves. High purity 1,2-dichloroethane and acetic acid were stored over 4Å molecular sieves. The Dess–Martin periodinane\(^{96}\) and dimethyldioxirane\(^{117}\) were prepared according to literature procedures. The molarity of \(n\)-butyllithium solutions was determined using \(N\)-benzylidenebenzylamine as an indicator (average of three determinations).\(^ {118}\) Phase-separated 1:1 mixtures of chlorotrimethylsilane and triethylamine were prepared by the addition of equal volume amounts of each compound via syringe to a
15 mL Pyrex test tube fitted with a rubber septum and the subsequent centrifugation of the mixture for 30 min.

**Instrumentation.** Infrared (IR) spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrophotometer internally referenced to a polystyrene standard. Data are presented as follows: frequency of absorption (cm\(^{-1}\)), intensity of absorption (s = strong, m = medium, w = weak, br = broad), and assignment (where appropriate). Proton nuclear magnetic resonance (\(^1\)H NMR) and carbon nuclear magnetic resonance (\(^{13}\)C NMR) spectra were obtained on General Electric QE-300 (300 MHz) or JEOL JX-400 (400 MHz) NMR spectrometers; chemical shifts are expressed in parts per million (δ scale) relative to an internal standard of chloroform (\(^1\)H, 7.26; \(^{13}\)C, 77.0), benzene-\(d_6\) (\(^1\)H, 7.20; \(^{13}\)C, 128.0), methanol-\(d_4\) (\(^{13}\)C, 44.9), acetone-\(d_6\) (\(^{13}\)C, 29.8), or acetonitrile-\(d_3\) (\(^{13}\)C, 1.3). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, ABq = AB quartet), integration, coupling constant in hertz (Hz), and assignment. \(^1\)H NMR difference nOe spectra of degassed samples were obtained on a Bruker AM-500 (500 MHz) NMR spectrometer. High resolution mass spectra (HRMS) were obtained at the University of California, Irvine Mass Spectrometry Facility.
Methylmagnesium bromide (30.0 mL of a 3.0 M solution in diethyl ether, 90.0 mmol, 2.1 equiv) was added to an ice-cooled solution of aldehyde 80 (10.3 g, 43.8 mmol, 1 equiv)\textsuperscript{75a} in tetrahydrofuran (400 mL). The reaction mixture was stirred at 0 °C for 30 min. After stirring for 5 min at 23 °C, the reaction mixture was partitioned between saturated aqueous ammonium chloride solution (275 mL) and ethyl acetate (100 mL). The layers were separated, and the product was extracted with ethyl acetate (2 × 300 mL). The combined organics were dried over sodium sulfate and were concentrated. The crude solid was diluted with toluene (400 mL) at 23 °C. p-Toluenesulfonic acid monohydrate (9.17 g, 48.2 mmol, 1.1 equiv) was added, and the resulting solution was stirred at 100 °C for 2 h. The reaction mixture was cooled to 23 °C and was partitioned between water (300 mL) and ethyl acetate (100 mL). The layers were separated, and the product was extracted with ethyl acetate (2 × 300 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The residue was purified by flash chromatography (30–45% ethyl acetate–hexanes) to yield phthalide 81 (6.94 g, 89%) as a yellow oil.

\[ \begin{align*} 
^1H \text{ NMR} (400 \text{ MHz, CDCl}_3), \delta: 
7.56 & (t, 1H, J = 7.8 \text{ Hz, aryl H} \text{ (m to COCH}_3)), \\
6.91 & (d, 1H, J = 7.3 \text{ Hz, aryl H}), \\
6.88 & (d, 1H, J = 8.3 \text{ Hz, aryl H}), \\
5.41 & (q, 1H, J = 6.6 \text{ Hz, CHCH}_3), \\
3.94 & (s, 3H, OCH}_3), \\
1.54 & (d, 3H, J = 6.6 \text{ Hz, CH}_3) 
\end{align*} \]

\[ \begin{align*} 
^{13}C \text{ NMR} (100 \text{ MHz, CDCl}_3), \delta: 
168.2 & (C=O), \\
158.3, 153.9, 136.1, 113.1, 113.0, \\
110.5, 76.3 & (CHCH}_3), \\
55.8 & (OCH}_3), \\
20.3 & (CH}_3) 
\end{align*} \]
FTIR (neat), cm\(^{-1}\):

2978 (m), 2931 (m), 1758 (s, C=O), 1605 (s),
1488 (s), 1310 (s), 1284 (s), 1246 (s), 1204 (s),
1081 (s), 1036 (s)

HRMS (EI):

Calc'd for C\(_{10}\)H\(_{10}\)O\(_3\) [M]\(^+\): 178.0630
Found: 178.0629

TLC (80% EtOAc in hexanes), \(R_f\): 0.60
Boron tribromide (92 mL of a 1.0 M solution in CH₂Cl₂, 92.0 mmol, 2.4 equiv) was transferred via cannula into a cooled (−78 °C) solution of phthalide 81 (6.94 g, 38.9 mmol, 1 equiv) in dichloromethane (225 mL). Upon complete addition, the cold bath was removed and the reaction mixture was stirred at 23 °C for 105 min. The reaction mixture was partitioned between water (350 mL) and dichloromethane (100 mL). The layers were separated, and the product was extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the crude mixture by flash chromatography (30% ethyl acetate–hexanes) provided 7-hydroxy-3-methylphthalide 82 (5.62 g, 88%) as a tan solid.

\[ \text{H NMR (300 MHz, CDCl}_3) \delta: \]

- 7.54 (t, 1H, \( J = 7.8 \) Hz, aryl H (m to COTBS)),
- 6.91 (d, 1H, \( J = 8.3 \) Hz, aryl H), 6.90 (d, 1H, \( J = 7.4 \) Hz, aryl H), 5.57 (q, 1H, \( J = 6.7 \) Hz, \( \text{CHCH}_3 \)), 1.63 (d, 3H, \( J = 6.7 \) Hz, \( \text{CH}_3 \))

\[ \text{C NMR (100 MHz, CDCl}_3) \delta: \]

- 171.7 (C=O), 156.3, 151.4, 136.8, 115.2, 112.7, 110.7, 79.0 (CH(CH₃)), 20.1 (CH₃)

FTIR (neat), cm⁻¹:

- 3542 (m, OH), 3419 (br, OH), 2936 (w), 1724 (s, C=O), 1632 (m), 1606 (m), 1476 (m), 1306 (m), 1205 (m), 1095 (m), 1044 (m)

HRMS (EI):

- Calc'd for C₉H₇O₃ [M]⁺: 164.0473
- Found: 164.0468

TLC (80% EtOAc in hexanes), \( R_f \): 0.74
tert-Butyldimethylsilyl chloride (2.86 g, 19.0 mmol, 3.0 equiv) was added to a solution of 7-hydroxy-3-methylphthalide 82 (1.04 g, 6.33 mmol, 1 equiv) and triethylamine (3.50 mL, 25.3 mmol, 4.0 equiv) in tetrahydrofuran (25.0 mL) 23 °C. 4-Dimethylaminopyridine (77 mg, 633 µmol, 0.1 equiv) was added to the reaction mixture and the resulting cloudy suspension was stirred for 110 min. The reaction mixture was poured into water (70 mL), and the product was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (5% ethyl acetate–hexanes) provided silyl-protected phthalide 83 as a white solid (1.43 g, 81% yield).

\[
\begin{align*}
\text{H NMR (300 MHz, CDCl}_3\text{), } &\delta: 7.48 (t, 1H, J = 7.8 \text{ Hz, aryl H (m to COTBS)}), \\
&6.94 (d, 1H, J = 7.6 \text{ Hz, aryl H}), 6.81 (d, 1H, J = 8.1 \text{ Hz, aryl H}), 5.39 (q, 1H, J = 6.6 \text{ Hz, CHCH}_3), 1.55 (d, 3H, J = 6.7 \text{ Hz, CH}_3), 1.02 (s, 9H, SiC(CH}_3)_3, 0.25 (s, 6H, Si(CH}_3)_2
\end{align*}
\]

\[
\begin{align*}
\text{C NMR (100 MHz, CDCl}_3\text{), } &\delta: 168.0 (C=O), 155.0, 153.6, 135.5, 120.1, 115.9, 113.9, 76.0 (CHCH}_3), 25.6 (SiC(CH}_3)_3, 20.6 (CH}_3), 18.4 (SiC(CH}_3)_3), -4.5 (Si(CH}_3)_2
\end{align*}
\]

\[
\begin{align*}
\text{FTIR (neat), cm}^{-1}: 2932 (m), 2859 (m), 1768 (s, C=O), 1604 (s), 1481 (s), 1302 (s), 1256 (m), 1237 (m), 1200 (m), 1028 (s), 985 (s), 838 (s)
\end{align*}
\]

\[
\begin{align*}
\text{HRMS (EI): Calc'd for } C_{14}H_{19}O_3Si [M - CH}_3]^+: 263.1103
\end{align*}
\]
Found: 263.1097

TLC (20% EtOAc in hexanes), $R_f$: 0.49
Potassium bis(trimethylsilyl)amide (700 µL of a 0.5 M solution in toluene, 350 µmol, 1.3 equiv) was added via syringe to a degassed, chilled (−78 °C) solution of phthalide 81 (52.8 mg, 280 µmol, 1.1 equiv) in tetrahydrofuran (4.0 mL). The resulting bright yellow reaction mixture was stirred at −78 °C for 25 min, and then a 1:1 (v/v) mixture of chlorotrimethylsilane and triethylamine was added (240 µL, ~942 µmol TMSCl, 3.5 equiv). The reaction mixture was warmed to −20 °C and was stirred for 3.0 min, at which point the solution decolorized. After cooling to −78 °C, the reaction mixture was treated with 1,4-benzoquinone (29.1 mg, 269 µmol, 1 equiv), and the reaction flask was transferred to an oil bath preheated to 50 °C. After 10 min, the reaction mixture was cooled to 23 °C and was poured into a half-saturated aqueous ammonium chloride solution (30 mL). The product was extracted from the aqueous layer with a 1:1 mixture of ethyl acetate and hexanes (3 × 40 mL). The combined organic layers were dried over sodium sulfate and were concentrated. 

\[ ^1H\text{NMR (C}_6\text{D}_6) \] analysis of the crude reaction mixture using an internal standard (trans-1,2-dichloroethylene) indicated a 75% yield of the endo Diels–Alder adduct 86. For characterization purposes, adduct 86 was purified by flash chromatography (20–30% ethyl acetate–hexanes) in low yield (26%).

\[ ^1H\text{NMR (300 MHz, CDCl}_3)\]:

- 7.14 (t, 1H, J = 8.2 Hz, aryl H (m to COCH₃)),
- 6.68 (d, 1H, J = 8.3 Hz, aryl H (o to COCH₃)),
- 6.58 (d, 1H, J = 7.3 Hz, aryl H (p to COCH₃)),
- 5.86 (ABq, 2H, J = 10.3 Hz, \( \Delta v = 29.9 \text{ Hz} \)),
- CH=CH, 3.77 (s, 3H, OCH₃), 3.30 (ABq, 2H,
$J = 9.2 \text{ Hz}, \Delta \nu = 19.7 \text{ Hz}, \text{CHCH}$), 1.91 (s, 3H, CH$_3$), 0.17 (s, 9H, OSi(CH$_3$)$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$: 195.9 (C=O), 193.6 (C=O), 154.2, 146.0, 138.9, 137.8, 130.4, 129.5, 112.0, 111.1, 109.8, 83.3 (CCH$_3$), 57.0, 56.8, 55.4, 16.9 (CH$_3$), 1.0 (Si(CH$_3$)$_3$)

FTIR (neat), cm$^{-1}$: 2960 (w), 1676 (s, C=O), 1600 (m), 1484 (m), 1357 (s), 1307 (s), 1252 (m), 1173 (m), 1138 (m), 1048 (m), 844 (s)

HRMS (FAB): Calc'd for C$_{19}$H$_{23}$O$_5$Si [M + H]$^+$: 359.1315

TLC (30% EtOAc in hexanes), $R_f$: 0.39

$^1$H nOe difference spectra for 86 are summarized below (500 MHz, CDCl$_3$):
Potassium bis(trimethylsilyl)amide (450 µL of a 0.5 M solution in toluene, 225 µmol, 1.2 equiv) was added via syringe to a degassed, cooled (−78 °C) solution of phthalide 83 (52.2 mg, 188 µmol, 1 equiv) in tetrahydrofuran (2.5 mL). The bright orange reaction mixture was stirred at −78 °C for 30 min. A 1:1 (v/v) mixture of chlorotrimethylsilane in triethylamine (143 µL, −563 µmol TMSCl, 3.0 equiv) was added. The reaction mixture was warmed to −20 °C for 1.5 min, at which point the solution decolorized. The reaction mixture was cooled to −78 °C, quinone monoketal 87 (69 mg, 225 µmol, 1.2 equiv)79 was added, and the reaction flask was immediately transferred to a pre-heated (55 °C) oil bath. After 1 h, the reaction mixture was cooled to 23 °C. A half-saturated aqueous sodium bicarbonate solution (5 mL) was added, and the product was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash column chromatography of the residue (5–10% ethyl acetate–hexanes) provided the endo Diels–Alder adducts 88 and 89 (1:1 inseparable mixture of diastereomers, 48.3 mg, 39% yield) as colorless oils, along with quinone monoketal 87 (26.4 mg, 38%).

Diels–Alder adducts 88 and 89:

\(^1\)H NMR (300 MHz, CDCl\(_3\)), δ:

7.31–7.42 (m, 12H, Ph), 7.20–7.28 (m, 8H, Ph),
6.96–7.09 (complex, 2H, aryl H (m to COTBS)),
6.84 (d, 1H, \(J = 7.0\) Hz, aryl H), 6.74 (d, 1H, \(J = 7.2\) Hz, aryl H), 6.59 (d, 1H, \(J = 8.1\) Hz, aryl
**$^1$H NMR (neat), cm$^{-1}$:**

H, 6.57 (d, 1H, $J = 8.2$ Hz, aryl H), 6.03 (dd, 1H, $J = 10.3$, 1.5 Hz, C(O)CH=CH), 5.89 (dd, 1H, $J = 10.3$, 1.5 Hz, C(O)CH=CH), 5.30 (d, 1H, $J = 10.2$ Hz, C(O)CH=CH), 5.27 (d, 1H, $J = 10.2$ Hz, C(O)CH=CH), 4.96 (d, 1H, $J = 8.7$ Hz, CHPh), 4.78 (d, 1H, $J = 8.4$ Hz, CHPh), 4.66 (d, 1H, $J = 8.9$ Hz, CHPh), 4.63 (d, 1H, $J = 8.6$ Hz, CHPh), 3.40 (dd, 1H, $J = 9.4$, 1.6 Hz, C(O)CH), 3.30 (d, 1H, $J = 9.4$ Hz, C(O)CH), 3.27 (d, 1H, $J = 8.1$ Hz, C(O)CH), 3.22 (dd, 1H, $J = 8.1$, 1.5 Hz, C(O)CH), 2.05 (s, 3H, CH$_3$), 1.94 (s, 3H, CH$_3$), 1.04 (s, 18H, SiC(CH$_3$)$_3$), 0.30 (s, 6H, Si(CH$_3$)$_2$), 0.27 (s, 6H, Si(CH$_3$)$_2$), 0.26 (s, 18H, Si(CH$_3$)$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$:

194.0 (C=O), 193.4 (C=O), 150.1, 149.9, 146.9 (2C), 142.9, 141.3, 136.1, 135.8, 135.4, 134.9, 132.4, 132.3, 126.3–129.3 (complex, multiple C: 129.3, 129.2, 129.1, 129.0, 128.8, 128.7, 128.4, 128.0, 127.5, 126.9, 126.4, 126.3), 118.4 (2C), 113.3, 113.0, 109.5 (2C), 104.2, 103.6, 86.5, 86.4, 85.6, 84.2, 82.2, 81.9, 57.1, 56.9, 56.6, 56.1, 26.0 (2 × SiC(CH$_3$)$_3$), 19.8 (CH$_3$), 19.0 (CH$_3$), 18.4 (2 × SiC(CH$_3$)$_3$), 1.6 (2 × Si(CH$_3$)$_2$), −4.1 (2 × Si(CH$_3$)$_2$), −4.2 (2 × Si(CH$_3$)$_3$)

**FTIR (neat), cm$^{-1}$:**

2931 (m), 2858 (w), 1679 (s, C=O), 1596 (m),
HRMS (Cl): Calc'd for C_{38}H_{47}O_{6}Si_{2} [M + H]^+: 655.2911
Found: 655.2908

TLC (30% EtOAc in hexanes), Rf: 0.72

^1H nOe difference spectra for the following related adduct (~2:1 mixture of diastereomers) are summarized below (500 MHz, C_{6}D_{6}):
Potassium bis(trimethylsilyl)amide (330 µL of a 0.5 M solution in toluene, 164 µmol, 1.6 equiv) was added via syringe to a degassed, cooled (−78 °C) solution of phthalide 83 (43 mg, 154 µmol, 1.5 equiv) in tetrahydrofuran (1.0 mL). The bright orange solution was stirred at −78 °C for 30 min. A 1:1 (v/v) mixture of chlorotrimethylsilane and triethylamine (115 µL, ~461 µmol TMSCl, 4.5 equiv) was added and the reaction mixture was warmed to −20 °C for 1.0 min, at which point the solution decolorized. After cooling the reaction mixture to −78 °C, a solution of enedione 90 (19.5 mg, 102 µmol, 1 equiv) in THF (0.5 mL) was added by syringe. The reaction flask was immediately transferred to an oil bath preheated to 60 °C. After 70 min, the reaction mixture was cooled to 23 °C. A half-saturated aqueous sodium bicarbonate solution (5 mL) was then added to the reaction mixture and the product was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The crude oil was purified by flash chromatography (2% ethyl acetate–hexanes) to give the endo Diels–Alder adduct 91 (33.0 mg, 60%).

\(^1\text{H} \text{NMR (300 MHz, } \text{C}_6\text{D}_6\text{), } \delta:\)

6.88 (t, 1H, aryl H (m to COTBS)), 6.73 (d, 1H, J = 7.2 Hz, aryl H (o to COTBS)), 6.63 (d, 1H, J = 8.1 Hz, aryl H (p to COTBS)), 3.35 (d, 1H, J = 10.2 Hz, C(O)CHCOTMS), 2.94 (d, 1H, J = 10.2 Hz, C(O)CHCCH₃), 2.27 (m, 1H, CH), 2.05 (s, 3H, CH₃), 1.80–1.96 (complex, 3H, CH), 1.35–1.52 (complex, 2H, CH), 1.42 (s,
$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$:

3H, CH$_3$C=CCH$_3$), 1.35 (s, 3H, CH$_3$C=CCH$_3$),
1.17 (s, 9H, Si(CH$_3$)$_3$), 0.46 (s, 9H, Si(CH$_3$)$_3$),
0.40 (s, 3H, Si(CH$_3$)$_2$), 0.25 (s, 3H, Si(CH$_3$)$_2$)

208.1 (C=O), 204.0 (C=O), 150.0, 147.4,
131.8, 129.5, 123.6, 122.1, 118.5, 112.6,
109.6, 82.4 (CCH$_3$), 58.0, 57.8, 48.3, 44.6,
29.7, 29.0, 25.9 (SiC(CH$_3$)$_3$), 18.9, 18.7, 18.3,
18.1, 1.4 (Si(CH$_3$)$_3$), −4.1 (Si(CH$_3$)$_2$), −4.4
(Si(CH$_3$)$_2$)

FTIR (neat), cm$^{-1}$:

2930 (m), 2859 (m), 1708 (s, C=O), 1596 (m),
1478 (s), 1355 (m), 1315 (s), 1252 (s), 992 (m),
845 (s)

HRMS (FAB):

Calc'd for C$_{30}$H$_{45}$O$_5$Si$_2$ [M + H]+: 541.2805
Found: 541.2801

TLC (1:2:7 EtOAc:PhCH$_3$:hexane),

$R_f$: 0.53

$^1$H nOe difference spectra for 91 are summarized below (500 MHz, C$_6$D$_6$):

![Diagram of molecule 91 with nOe differences indicated]
A solution of hydrogen fluoride•pyridine buffered in pyridine was prepared at 0 °C by the addition of 70% hydrogen fluoride•pyridine (1.0 mL) to a solution of pyridine (4.0 mL) in THF (10.0 mL). A 200 µL-aliquot was added to an ice-cooled solution of adduct 91 (33.0 mg, 61.0 µmol, 1 equiv) in tetrahydrofuran (2.0 mL). The reaction mixture was stirred at 23 °C. After 1 h, another 200 µL-aliquot of hydrogen fluoride•pyridine solution was added. The reaction mixture was poured into water (5 mL) after 2 h and the product was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography purification of the residue (10–20% ethyl acetate–hexanes) provided tetracyclic compound 92 (13 mg, 60%) as a yellow solid.

\[1^1H\text{ NMR (300 MHz, C}_6\text{D}_6), \delta: 15.06 (s, 1H, enol H), 12.31 (s, 1H, phenol H), 7.51 (d, 1H, } J = 7.7 \text{ Hz, aryl } H (p \text{ to } COH), 7.16 (t, 1H, } J = 7.9 \text{ Hz, aryl } H (m \text{ to } COH), 6.93 (d, 1H, } J = 7.9 \text{ Hz, aryl } H (o \text{ to } COH), 5.14 (s, 1H, OH), 3.12 (s, 1H, CHC(OH)CH}_3), 2.59 (d, 1H, } J = 16.9 \text{ Hz, } H_b), 2.49 (m, 1H, } H_c), 2.05 (m, 1H, } H_a), 1.90 (dd, 1H, } J = 16.5, 4.9 \text{ Hz, } H_d), 1.38–1.59 (complex, 2H, } H_c), 1.52 (s,}
\[ \text{\(^{13}\text{C} \text{NMR (100 MHz, CDCl}_3\), } \delta:\]

\[
213.0 \text{ (C=O)}, \ 191.9 \text{ (C=O)}, \ 181.9 \text{ (C=O)}, \\
161.9, \ 148.7, \ 137.2, \ 124.1, \ 122.3, \ 116.8, \\
115.0, \ 112.9, \ 101.1, \ 72.8, \ 52.1, \ 47.7, \ 39.0, \\
32.0, \ 28.2, \ 27.6, \ 18.9 (2C)
\]

\[
\text{FTIR (neat), cm}^{-1}:
3457 \text{ (br, OH)}, \ 2922 \text{ (w)}, \ 1698 \text{ (w, C=O), 1614 (s), 1583 (s), 1454 (m), 1363 (m), 1312 (m), 1243 (m), 1206 (m), 1160 (m)}
\]

\[
\text{HRMS (FAB):}
\text{Calc'd for } \text{C}_{21}\text{H}_{23}\text{O}_{5} [M + H]^+ : 355.1545 \\
\text{Found: 355.1539}
\]

\[
\text{TLC (20\% EtOAc in hexanes), } R_f : \ 0.40
\]

\[
\text{\(^1H \text{nOe difference spectra for 92 are summarized below (500 MHz, C}_{6}\text{D}_6\):}
\]
Titanium(IV) isopropoxide (17.2 mL, 58.3 mmol, 5.0 equiv) was added to a degassed, cooled (−20 °C) solution of diisopropyl L-tartrate (14.7 mL, 69.9 mmol, 6.0 equiv) and crushed, activated 4Å molecular sieves (441 mg, 30% wt/wt) in dichloromethane (110 mL). 2-(Hydroxymethyl)-2-cyclohexen-1-one (1.47 g, 11.7 mmol, 1 equiv) was added to the reaction mixture by syringe in a dichloromethane solution (2 × 5.0 mL portions), and the resulting reaction mixture was stirred for 30 min at −20 °C. tert-Butyl hydroperoxide (TBHP, 14.6 mL of a 4.0 M solution in CH₂Cl₂, 58.3 mmol, 5.0 equiv) was added dropwise to the reaction mixture over a period of 20 min. To drive the reaction to completion, more TBHP (7.3 mL, 29.2 mmol, 2.5 equiv) was added after 13.5 h. After 15 h, the reaction mixture was removed from the cooling bath, a solution of 10% aqueous tartaric acid (100 mL) was added, and the resulting solution was stirred vigorously at 23 °C. The mixture was filtered through a pad of Celite and rinsed with water (200 mL) and dichloromethane (250 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (200 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography (40–50% ethyl acetate–hexanes) of the crude oil provided the chiral epoxide 94 as a pale yellow oil (1.23 g, 74%).

Acylation of epoxide 94 (20.1 mg, 141 µmol, 1 equiv) proceeded with optically-pure α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride (~353 µmol, 2.5 equiv), triethylamine (79 µL, 566 µmol, 4.0 equiv), and 4-dimethylaminopyridine (cat.) in dichloromethane (2.0 mL) at 23 °C for 4 h. Analysis of the purified Mosher ester derivative by ¹H NMR (400 MHz, CDCl₃) established that epoxide 94 was of ≥94% ee. The Mosher acid chloride (~353 µmol, 2.5 equiv) was prepared in situ from chiral α-methoxy-α-(trifluoromethyl)phenylacetic acid (83.0 mg, 353 µmol, 2.5 equiv), oxalyl
chloride (49 µL, 566 µmol, 4.0 equiv), and N,N-dimethylformamide (2.0 µL, 26 µmol, 0.18 equiv) in dichloromethane (1.0 mL), followed by removal of the excess oxalyll chloride and solvent at reduced pressure (1 Torr).

$^1$H NMR (300 MHz, CDCl$_3$), δ: 3.62 (br t, 1H, epoxide H), 2.55 (dt, 1H, $J = 16.9$, 4.7 Hz, C(O)CH$_2$), 3.62 (br t, 1H, epoxide H), 2.22–2.29 (m, 1H, C(O)CH$_2$), 1.62–2.13 (complex, 4H, CH$_2$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ: 207.6 (C=O), 60.6 (quaternary epoxide C), 60.5, 59.9, 36.9, 23.0, 17.6

FTIR (neat), cm$^{-1}$: 3415 (br, OH), 2948 (m), 1704 (s, C=O), 1432 (w), 1351 (w), 1101 (w), 1047 (m), 879 (w)

HRMS (EI): Calc'd for C$_7$H$_9$O$_3$ [M − H]$^+$: 141.0552

Found: 141.0554

TLC (EtOAc), $R_f$: 0.56
Triethylamine (2.5 mL, 17.9 mmol, 2.5 equiv) was added to an ice-cooled solution of tert-butyldimethylsilyl chloride (2.16 g, 14.4 mmol, 2.0 equiv) and epoxide 94 (1.02 g, 7.18 mmol, 1 equiv) in dichloromethane (40 mL). 4-Dimethylaminopyridine (219 mg, 1.79 mmol, 0.25 equiv) was added and the reaction mixture was stirred at 23 °C for 3 h. The reaction mixture was poured into water (60 mL) and the product was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (3–5% ethyl acetate–hexanes) gave TBS-protected epoxide 95 (1.67 g, 91%) as a colorless oil.

\[ \begin{align*}
\text{TBSCl, } & \text{Et}_3\text{N, DMAP} \\
\text{CH}_2\text{Cl}_2, & \text{23 °C} \\
\end{align*} \]

94 \rightarrow 95

91%

\[ \text{TBSCI, } \text{Et}_3\text{N, DMAP} \]

\[ \text{CH}_2\text{Cl}_2, \text{23 °C} \]

\[ \text{91%} \]

\[ \begin{align*}
\text{94} & \rightarrow \text{95} \\
\end{align*} \]

\[ \begin{align*}
\text{H NMR (300 MHz, CDCl}_3), \delta: & \quad 4.03 \text{ (ABq, 2H, } J = 12.4 \text{ Hz, } \Delta v = 92.6 \text{ Hz, CH}_2\text{OTBS}), 3.70 \text{ (br t, 1H, epoxide H), 2.56 (dt, 1H, } J = 15.9, 4.5 \text{ Hz, C(O)CH}_2), 2.20–2.25 \text{ (m, 1H, C(O)CH}_2), 1.60–2.07 \text{ (complex, 4H, CH}_2), 0.87 \text{ (s, 9H, SiC(CH}_3)_3), 0.05 \text{ (s, 6H, Si(CH}_3)_2) \\
\end{align*} \]

\[ \begin{align*}
\text{C NMR (100 MHz, CDCl}_3), \delta: & \quad 205.3 \text{ (C=O), 60.5 (quaternary epoxide C), 58.5, 57.7, 36.7, 25.5 (SiC(CH}_3)_3), 22.8, 17.9, 17.3, -5.8 \text{ (Si(CH}_3)_2), -5.9 \text{ (Si(CH}_3)_2) \\
\end{align*} \]

\[ \begin{align*}
\text{FTIR (neat), cm}^{-1}: & \quad 2931 \text{ (s), 2857 (m), 1710 (s, C=O), 1463 (m), 1393 (w), 1255 (m), 1134 (m), 1068 (m), 875 (m), 839 (s), 780 (m) } \\
\end{align*} \]

\[ \begin{align*}
\text{HRMS (Cl):} & \quad \text{Calc’d for C}_{13}\text{H}_{25}\text{O}_3\text{Si [M + H]}^+: 257.1573 \quad \text{Found: 257.1569} \\
\end{align*} \]

\[ \begin{align*}
\text{TLC (40% EtOAc in hexanes), } R_f: & \quad 0.81 \\
\end{align*} \]
n-Butyllithium (1.17 mL of a 2.52 M solution in hexanes, 2.95 mmol, 1.50 equiv) was added to a degassed, cooled (-78 °C) solution of diisopropylamine (420 µL, 2.99 mmol, 1.52 equiv) in tetrahydrofuran (8.5 mL). The resulting solution was stirred at 0 °C for 10 min and was then cooled to -78 °C. Chlorotrimethylsilane (750 µL, 5.90 mmol, 3.0 equiv) was added to the reaction mixture, followed by the dropwise addition of a solution of ketone 95 (504 mg, 1.97 mmol, 1 equiv) in THF (1.5 mL). After 45 min, the reaction mixture was quenched with the addition of triethylamine (825 µL, 5.92 mmol, 3.0 equiv) and was poured into saturated aqueous sodium bicarbonate solution (10 mL). The aqueous layer was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 x 8 mL). The combined organic layers were dried over sodium sulfate and were concentrated.

The resulting crude oil was dissolved in dichloromethane (5.0 mL) and was cooled to 0 °C. Phenylselenenyl chloride (418 mg, 2.18 mmol, 1.1 equiv) was added and the reaction mixture was stirred at 0 °C. After 15 min, a 1:9 mixture of saturated aqueous sodium bicarbonate solution and water (10 mL) was added to the reaction mixture, which was then stirred at 23 °C. The layers were separated, and the aqueous layer was further extracted with a 1:1 mixture of ethyl acetate and hexanes (2 x 10 mL). The combined organic layers were dried over sodium sulfate and were concentrated.

The resulting crude oil was diluted with tetrahydrofuran (10.0 mL) and was cooled to 0 °C. Acetic acid (23 µL, 394 µmol, 0.2 equiv) and hydrogen peroxide (469 mg of a 30% (w/w) aqueous solution, 4.14 mmol, 2.1 equiv) were added sequentially to the reaction mixture, which was then stirred vigorously at 23 °C. After 15 min, the reaction mixture was diluted with a 1:9 mixture of saturated aqueous sodium bicarbonate solution and water (10 mL), brine (2 mL), and ethyl acetate (8 mL). The layers were separated, and
the aqueous layer was further extracted with ethyl acetate (2 x 10 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the crude oil by flash chromatography (5–10% ethyl acetate–hexanes) provided enone 96 as a tan oil (361 mg, 72%).

\[ \text{H NMR (400 MHz, CDCl}_3\text{), } \delta: \]

- 6.52 (dm, 1H, \( J = 10.6 \) Hz, C(O)CH=CH)
- 5.93 (dt, 1H, \( J = 10.6, 2.0 \) Hz, C(O)CH)
- 4.08 (ABq, 2H, \( J = 12.6 \) Hz, \( \Delta v = 80.7 \) Hz, CH₄OTBS)
- 3.82 (br t, 1H, epoxide H)
- 2.92 (dd, 1H, \( J = 21.6, 4.4 \) Hz, CH₂)
- 2.68 (dq, 1H, \( J = 21.4, 2.5 \) Hz, CH₂)
- 0.83 (s, 9H, SiC(CH₃)₃)
- 0.02 (s, 6H, Si(CH₃)₂)

\[ \text{C NMR (100 MHz, CDCl}_3\text{), } \delta: \]

- 195.0 (C=O)
- 143.2 (C(O)CH=CH)
- 126.8 (C(O)CH)
- 60.3 (quaternary epoxide C)
- 58.1, 54.8, 27.3, 25.7 (SiC(CH₃)₃)
- 18.1 (SiC(CH₃)₃)
- -5.5 (Si(CH₃)₂)
- -5.6 (Si(CH₃)₂)

FTIR (neat), cm⁻¹:

- 2931 (m), 2857 (m), 1679 (s, C=O), 1470 (w), 1407 (m), 1256 (m), 1141 (m), 1082 (s), 837 (s), 780 (m)

HRMS (Cl):

- Calc'd for \( C_{13}H_{23}O_3Si \) [M + H]⁺: 255.1416
- Found: 255.1417

TLC (10% EtOAc in hexanes), \( R_f: \)

- 0.22
Potassium bis(trimethylsilyl)amide (430 µL of a 0.5 M solution in toluene, 214 µmol, 5.5 equiv) was added to a degassed, chilled (−78 °C) solution of phthalide 81 (36.1 mg, 203 µmol, 5.2 equiv) in tetrahydrofuran (2.5 mL). The resulting bright yellow solution was stirred at −78 °C for 25 min, at which point a 1:1.6 (v/v) mixture of chlorotrimethylsilane and triethylamine (85 µL, ~250 µmol TMSCl, 13.0 equiv) was added. The reaction mixture was warmed to −20 °C for 6.0 min (solution decolorized) and was then cooled to −78 °C. Enone 96 (10 mg, 39 µmol, 1 equiv) was added to the reaction mixture in a tetrahydrofuran solution (0.5 mL). The reaction flask was immediately transferred to an oil bath preheated to 50 °C. After 40 min, the reaction mixture was concentrated in vacuo and then partitioned between a half-saturated aqueous sodium bicarbonate solution (40 mL) and a 1:1 mixture of ethyl acetate and hexanes (50 mL). The layers were separated and the aqueous layer was further extracted with a 1:1 mixture of ethyl acetate in hexanes (50 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography of the residue (5% ethyl acetate–hexanes) provided the endo Diels-Alder adduct 98 (15 mg) in 75% yield.

$^1$H NMR (300 MHz, CDCl$_3$), δ:

7.09 (t, 1H, $J = 8.2$ Hz, aryl H (m to COCH$_3$)),
6.84 (dd, 1H, $J = 10.1$, 4.8 Hz, C(O)CH=CH),
6.75 (d, 1H, $J = 7.3$ Hz, aryl H (p to COCH$_3$)),
6.63 (d, 1H, $J = 8.2$ Hz, aryl H (o to COCH$_3$)),
5.63 (dd, 1H, $J = 10.2$, 1.2 Hz, C(O)CH), 3.75 (s, 3H, OCH$_3$), 3.69 (s, 1H, OH), 3.43 (ABq,
$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$: 195.7 (C=O), 154.3, 147.4, 145.8, 129.3, 129.2, 127.0, 113.8, 111.3, 109.9, 82.8, 78.0, 72.2, 55.0, 51.5, 50.1, 25.7 (SiC(CH$_3$)$_3$), 19.3, 18.1, 1.0 (Si(CH$_3$)$_3$), -5.5 (Si(CH$_3$)$_2$), -5.6 (Si(CH$_3$)$_2$)

FTIR (neat), cm$^{-1}$: 3459 (w, OH), 2932 (m), 2857 (m), 1693 (m, C=O), 1599 (m), 1484 (m), 1309 (s), 1252 (s), 1131 (m), 1053 (m), 987 (w), 840 (s), 757 (m)

HRMS (FAB): Calc'd for C$_{26}$H$_{41}$O$_6$Si$_2$ [M + H]$^+$: 505.2441

Found: 505.2446

TLC (30% EtOAc in hexanes), $R_f$: 0.79

$^1$H nOe difference spectra for 98 are summarized below (500 MHz, CDCl$_3$):
Ethyl 2-cyclohexanonecarboxylate (150 µL, 938 mmol, 1 equiv) was added to a suspension of sodium hydride (43.1 mg of a 60% dispersion in mineral oil, 1.03 mmol, 1.1 equiv) in tetrahydrofuran (10.0 mL) at 23 °C. After 10 min, the reaction mixture was cooled to -78 °C and a solution of dimethyldioxirane (DMDO, 17.0 mL of a 0.086 M solution in acetone, 1.46 mmol, 1.6 equiv) was added. The reaction mixture was quenched after 30 min upon addition of a pH 7 phosphate buffer (10 mL) and was warmed to 23 °C. The reaction mixture was poured into water (50 mL) and the product was extracted with ethyl acetate (3 × 60 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (10% ethyl acetate–hexanes) provided alcohol **99** (156 mg, 89%) as a colorless oil.

**1H NMR (300 MHz, C₆D₆), δ:**
4.60 (s, 1H, OH), 3.94 (m, 2H, OCH₂CH₃), 2.64 (m, 1H, CH₂), 2.44 (dt, 1H, J = 13.8, 4.6 Hz, CH₂), 2.24 (m, 1H, CH₂), 1.38–1.55 (complex, 4H, CH₂), 1.21 (m, 1H, CH₂), 0.90 (t, 3H, J = 7.1 Hz, OCH₂CH₃)

**13C NMR (100 MHz, CDCl₃), δ:**
207.2 (C=O), 170.0 (CO₂Et), 80.6 (COH), 62.0 (OCH₂CH₃), 38.8, 37.6, 27.0, 21.9, 13.9

**FTIR (neat), cm⁻¹:**
3458 (br, OH), 2944 (m), 1721 (s, C=O), 1450 (w), 1368 (w), 1250 (s), 1211 (m), 1115 (m), 1018 (m)

**HRMS (EI):**
Calc'd for C₉H₁₄O₄ [M]⁺: 186.0892
Found: 186.0894

**TLC (20% EtOAc in hexanes), Rf:** 0.32
1. LOA, THF, -78 °C
2. TMSCl:Et₃N, -78→23 °C
3. PhSeCl, CH₂Cl₂, 0 °C
4. H₂O₂, pyridine, CH₂Cl₂, 0 °C

n-Butyllithium (250 µL of a 2.37 M solution in hexanes, 590 µmol, 2.2 equiv) was added to a degassed, cooled (−78 °C) solution of diisopropylamine (80 µL, 620 µmol, 2.3 equiv) in tetrahydrofuran (3 mL). The resulting solution was warmed to 0 °C for 10 min and was cooled to −78 °C. A solution of alcohol 99 (50 mg, 270 µmol, 1 equiv) in THF (0.5 mL) was added to the reaction mixture. After 65 min, the reaction mixture was quenched upon addition of a 1:1 (v/v) mixture of chlorotrimethylsilane and triethylamine (205 µL, ~810 µmol TMSCl, 3.0 equiv). After 5 min, the reaction mixture was allowed to warm slowly to 23 °C over 45 min. The reaction mixture was poured into a 1:9 mixture of saturated aqueous sodium bicarbonate solution and water (10 mL), and the product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 × 25 mL). The combined organic layers were dried over sodium sulfate and were concentrated.

A solution of the resulting crude oil in dichloromethane (2.5 mL) was cooled to 0 °C. Phenylselenenyl chloride (62 mg, 320 µmol, 1.2 equiv) in dichloromethane (0.5 mL) was added to the reaction mixture. After 20 min, the reaction mixture was diluted with a 1:9 mixture of saturated aqueous sodium bicarbonate solution and water (4 mL) and a 1:1 mixture of ethyl acetate and hexanes (3 mL) and was warmed to 23 °C. The layers were separated and the organic layer was washed with a 1:9 mixture of saturated aqueous sodium bicarbonate solution and water (4 mL). The organic layer was dried over sodium sulfate and was concentrated.

The crude α-selenide was diluted with dichloromethane (2.0 mL) and was cooled to 0 °C. Pyridine (39 µL, 540 µmol, 2.0 equiv) and a solution of hydrogen peroxide (94 mg of a 30% (w/w) aqueous solution, 830 µmol, 3.1 equiv) in water (2 mL) were added sequentially to the reaction mixture. After 40 min, the reaction mixture was warmed to 23
The reaction mixture was then poured into saturated aqueous sodium bicarbonate solution (7 mL), and the product was extracted with dichloromethane (3 × 5 mL). The combined organic layers were washed with water (5 mL), dried over sodium sulfate, and were concentrated. Flash chromatography of the crude oil (5% ethyl acetate–hexanes) afforded enone 100 (36 mg, 52%) as a pale yellow oil.

$^1$H NMR (300 MHz, C$_6$D$_6$), δ: 6.17 (dt, 1H, $J = 10.2$, 3.8 Hz, C(O)CH=CH), 5.95 (dt, 1H, $J = 10.2$, 1.9 Hz, C(O)CH), 3.95 (q, 2H, $J = 7.1$ Hz, OCH$_2$CH$_3$), 2.38 (m, 1H, CH$_2$), 1.75–2.00 (complex, 3H, CH$_2$), 0.92 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH$_3$), 0.44 (s, 9H, Si(CH$_3$)$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ: 194.3 (C=O), 171.0 (CO$_2$Et), 150.1 (C(O)CH=CH), 127.9 (C(O)CH), 80.3 (COTMS), 61.4, 35.0, 23.6, 13.9, 1.6 (Si(CH$_3$)$_3$)

FTIR (neat), cm$^{-1}$: 2958 (m), 1754 (s, C=O), 1694 (s, C=O), 1388 (m), 1249 (s), 1180 (s), 1130 (s), 898 (m), 846 (s)

HRMS (Cl): Calc'd for C$_{12}$H$_{21}$O$_4$Si [M + H]$^+$: 257.1209

Found: 257.1202

TLC (20% EtOAc in hexanes), $R_f$: 0.50
Potassium bis(trimethylsilyl)amide (410 µL of a 0.5 M solution in toluene, 206 µmol, 1.4 equiv) was added to a degassed, chilled (−78 °C) solution of phthalide 81 (34.1 mg, 191 µmol, 1.3 equiv) in tetrahydrofuran (2.0 mL). The resulting bright yellow solution was stirred at −78 °C for 35 min. A 1:1 (v/v) mixture of chlorotrimethylsilane and triethylamine (170 µL, −662 µmol TMSCI, 4.5 equiv) was added and the reaction mixture was warmed to −20 °C for 1 min, at which point the solution decolorized. The reaction mixture was cooled to −78 °C, and a solution of enone 100 (37.7 mg, 147 µmol, 1 equiv) in tetrahydrofuran (500 µL) was added. The reaction flask was transferred to a pre-heated (55 °C) oil bath for 65 min. The cooled reaction mixture was diluted with a half-saturated aqueous sodium bicarbonate solution (5 mL), and the product was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography (2–5% ethyl acetate–hexanes) of the residue provided the exo Diels–Alder adduct 101 (11.0 mg, 15%), the exo Diels–Alder adduct 102 (7.0 mg, 9%), and the enone 100 (16.5 mg, 44%). Adduct 102 was contaminated with a minor amount of the apparent endo Diels–Alder diastereomeric adducts.
Major adduct 101:

$^1$H NMR (300 MHz, CDCl$_3$), $\delta$: 7.17 (dd, 1H, $J = 8.1, 7.4$ Hz, aryl H (m to COCH$_3$)), 6.77 (d, 1H, $J = 7.3$ Hz, aryl H), 6.72 (d, 1H, $J = 8.2$ Hz, aryl H), 4.23 (q, 2H, $J = 7.1$ Hz, OCH$_2$CH$_3$), 3.86 (s, 3H, OCH$_3$), 2.54 (m, 1H, CH$_2$), 2.50 (d, 1H, $J = 8.4$ Hz, C(O)CH), 2.38 (m, 1H, CH$_2$), 2.10 (m, 1H, CH$_2$), 1.96 (d, 1H, $J = 7.3$ Hz, C(O)CHCH), 1.93 (m, 1H, CH$_2$), 1.73 (s, 3H, CH$_3$), 1.30 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH$_3$), 0.13 (s, 9H, Si(CH$_3$)$_3$), 0.12 (s, 9H, Si(CH$_3$)$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$: 206.4 (C=O), 172.0 (CO$_2$Et), 152.5, 151.2, 132.5, 129.2, 110.2 (2C), 108.9, 82.2, 80.6, 61.5, 56.4, 55.3, 43.4, 32.6, 17.4, 15.6, 14.1, 1.7 (Si(CH$_3$)$_3$), 0.8 (Si(CH$_3$)$_3$)

FTIR (neat), cm$^{-1}$: 2958 (m), 1754 (m, C=O), 1709 (w, C=O), 1600 (w), 1483 (m), 1314 (m), 1250 (s), 1152 (m), 1054 (m), 845 (s)

HRMS (FAB): Calc'd for C$_{25}$H$_{39}$O$_7$Si$_2$ [M + H]$^+$: 507.2234

Found: 507.2229

TLC (20% EtOAc in hexanes), $R_f$: 0.61

Minor adduct 102:

$^1$H NMR (300 MHz, CDCl$_3$), $\delta$: 7.17 (t, 1H, $J = 7.8$ Hz, aryl H (m to COCH$_3$)), 6.75 (d, 1H, $J = 7.4$ Hz, aryl H), 6.72 (d, 1H, $J = 8.4$ Hz, aryl H), 4.17 (q, 2H, $J = 7.5$ Hz, OCH$_2$CH$_3$), 3.86 (s, 3H, OCH$_3$), 2.54 (d, 1H, $J$
= 8.3 Hz, C(O)CH), 2.41 (q, 1H, J = 7.2 Hz, CH₂), 2.24 (m, 2H, C(O)CHCH, CH₂), 2.08 (m, 1H, CH₂), 1.79 (m, 1H, CH₂), 1.70 (s, 3H, CH₃), 1.23 (t, 3H, J = 7.6 Hz, OCH₂CH₃), 0.20 (s, 9H, Si(CH₃)₃), 0.13 (s, 9H, Si(CH₃)₃)

¹³C NMR (100 MHz, CDCl₃), δ:
205.2 (C=O), 172.7 (CO₂Et), 152.8, 151.5, 131.9, 129.3, 110.1, 110.0, 108.8, 82.3, 80.7, 61.4, 56.9, 55.3, 44.0, 33.4, 18.4, 16.1, 14.0, 2.1 (Si(CH₃)₃), 0.8 (Si(CH₃)₃)

FTIR (neat), cm⁻¹: 2958 (w), 1753 (m, C=O), 1716 (w, C=O), 1599 (w), 1483 (m), 1312 (m), 1249 (s), 1174 (m), 1053 (m), 844 (s)

HRMS (FAB): Calc'd for C₂₅H₃₉O₇Si₂ [M + H]⁺: 507.2234
Found: 507.2229

TLC (20% EtOAc in hexanes), Rf: 0.51
Potassium bis(trimethylsilyl)amide (405 µL of a 0.5 M solution in toluene, 200 µmol, 5.2 equiv) was added to a degassed, chilled (−78 °C) solution of phthalide 81 (34.9 mg, 196 µmol, 5.0 equiv) in tetrahydrofuran (2.5 mL). The resulting bright yellow solution was stirred for 25 min at −78 °C, at which point a solution of enone 96 (10 mg, 39 µmol, 1 equiv) in tetrahydrofuran (0.5 mL) was added. The reaction mixture was allowed to warm to 23 °C. After 55 min, the reaction mixture was quenched with the addition of a pH 7 phosphate buffer (4 mL), water (2 mL), and dichloromethane (5 mL). The layers were separated, and the aqueous layer was further extracted with dichloromethane (2 × 6 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography (20–25% ethyl acetate–hexanes) of the residue gave the tricyclic product 103 (6.4 mg, 38%) as a tan oil.

$^1$H NMR (400 MHz, C$_6$D$_6$), δ: 17.14 (s, 1H, enol H), 7.26 (dd, 1H, $J = 7.8$, 1.0 Hz, aryl H), 7.14 (t, 1H, $J = 8.0$ Hz, aryl H (m to COCH$_3$)), 6.41 (d, 1H, $J = 7.8$ Hz, aryl H), 4.43 (ABq, 2H, $J = 12.7$ Hz, $\Delta\nu = 97.2$ Hz, CH$_2$OTBS), 3.85 (d, 1H, $J = 3.5$ Hz, epoxide H), 3.36 (s, 3H, OCH$_3$), 2.77 (dd, 1H, $J = 12.0$, 6.0 Hz, CHC(OH)CH$_3$), 2.18 (dm, 1H, $J = 14.6$ Hz, CH$_2$), 1.46 (dd, 1H, $J = 14.2$, 12.2 Hz, CH$_2$), 1.02 (s, 9H, Si(CH$_3$)$_3$), 1.01 (s, 3H, CH$_3$), 0.14 (s, 3H, Si(CH$_3$)$_2$), 0.12 (s, 3H, Si(CH$_3$)).
\(^{13}\)C NMR (100 MHz, CDCl\(_3\)), \(\delta\):

\begin{align*}
187.8 \text{ (C=O)}, & \quad 179.2 \text{ (C=O)}, \quad 159.5, \quad 151.7, \\
134.4, & \quad 117.0, \quad 115.1, \quad 111.5, \quad 105.3, \quad 73.1, \quad 58.3, \\
58.2, & \quad 58.1, \quad 56.3, \quad 39.0, \quad 25.9 \text{ (SiC(CH}_3)_3), \quad 24.1, \\
21.8, & \quad 18.4, \quad -5.3 \text{ (Si(CH}_3)_2), \quad -5.4 \text{ (Si(CH}_3)_2) \\
\end{align*}

FTIR (neat), cm\(^{-1}\):

\begin{align*}
3466 \text{ (br, OH)}, & \quad 2930 \text{ (m)}, \quad 2856 \text{ (w)}, \quad 1595 \text{ (s, C=O)}, \quad 1470 \text{ (m)}, \quad 1337 \text{ (m)}, \quad 1268 \text{ (s)}, \quad 1127 \text{ (m)}, \\
1046 \text{ (m)}, & \quad 839 \text{ (s)} \\
\end{align*}

HRMS (FAB): 

Calc'd for C\(_{23}\)H\(_{33}\)O\(_6\)Si [M + H]: 433.2046

Found: 433.2043

TLC (30% EtOAc in hexanes), \(R_f\): 0.64

\(^1\)H nOe difference spectra for 103 are summarized below (500 MHz):

\[ \text{in C}_6\text{D}_6: \]

\[ \text{in CDCl}_3: \]
Sodium azide (486 mg, 7.47 mmol, 1.1 equiv) was added to a solution of 6-chloromethyl-2,2-dimethyl-1,3-dioxin-4-one (1.84 g, 10.4 mmol, 1 equiv) in N,N-dimethylformamide (5.0 mL) at 23 °C. The reaction mixture was stirred for 35 min and was then poured into water (50 mL). The product was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (20% ethyl acetate–hexanes) provided azide 111 (1.17 g) in 94% yield as a pale yellow oil.

\[
\begin{align*}
\text{H NMR (300 MHz, CDCl}_3\text{), } &\delta: \\
5.46 &\text{ (s, 1H, C(O)CH), 3.86 (s, 2H, CH}_2\text{N}_3\text{),} \\
1.70 &\text{ (s, 6H, CH}_3\text{)}
\end{align*}
\]

\[
\begin{align*}
\text{C NMR (100 MHz, CDCl}_3\text{), } &\delta: \\
164.7 &\text{ (C(O)CH=C), 160.0 (C=O), 107.4} \\
94.6 &\text{ (C(O)CH), 50.6 (CH}_2\text{N}_3\text{), 24.8} \\
9.6 &\text{(CH}_3\text{)}
\end{align*}
\]

FTIR (neat), cm\(^{-1}\): 
3000 (w), 2109 (s, N\(_3\)), 1732 (s, C=O), 1643
(m), 1392 (m), 1273 (m), 1204 (m), 1018 (m),
902 (m), 812 (m)

HRMS (EI):
Calc’d for C\(_7\)H\(_9\)N\(_3\)O\(_3\) [M]: 183.0644
Found: 183.0644

TLC (30% EtOAc in hexanes), \(R_f\):
0.64
Triphenylphosphine (1.84 g, 7.03 mmol, 1.1 equiv) was slowly added in two portions to a solution of azide 111 (1.17 g, 6.39 mmol, 1 equiv) in tetrahydrofuran (5.0 mL). After 5 min, water (175 µL, 9.58 mmol, 1.5 equiv) was added to the reaction mixture. The reaction mixture was stirred at 23 °C for 36 h and was then concentrated in vacuo. Flash chromatography (3% methanol–dichloromethane) of the crude oil afforded amine 112 (918 mg, 92%) as an orange oil.

\[
\begin{align*}
\text{Triphenylphosphine} & \; (1.84 \text{ g}, \; 7.03 \text{ mmol}, \; 1.1 \text{ equiv}) \\
& \; \text{was slowly added in two portions to a solution of azide 111} \; (1.17 \text{ g}, \; 6.39 \text{ mmol}, \; 1 \text{ equiv}) \; \text{in tetrahydrofuran (5.0 mL). After 5 min, water (175 µL, 9.58 mmol, 1.5 equiv) was added to the reaction mixture. The reaction mixture was stirred at 23 °C for 36 h and was then concentrated in vacuo. Flash chromatography (3% methanol–dichloromethane) of the crude oil afforded amine 112 (918 mg, 92%) as an orange oil.}
\end{align*}
\]
Sodium triacetoxyborohydride (4.95 g, 23.4 mmol, 4.0 equiv) and formaldehyde (1.75 g of a 37% (w/w) aqueous solution, 17.5 mmol, 3.0 equiv) were added sequentially to a solution of amine 112 (918 mg, 5.84 mmol, 1 equiv) in 1,2-dichloroethane (40.0 mL) at 23 °C (it was important to keep the reaction mixture at a concentration of ≤0.15 M). After 45 min, the reaction mixture was poured into saturated aqueous sodium bicarbonate solution (30 mL), and the product was extracted with dichloromethane (3 × 30 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography of the crude oil (30% ethyl acetate–hexanes with 1% triethylamine) gave dimethylamine 110 (822 mg, 76%) as a pale yellow solid. The 13C NMR spectral assignments were confirmed by an APT experiment.

1H NMR (400 MHz, CDCl3), δ: 5.43 (s, 1H, C(O)CH), 3.00 (s, 2H, CH₂N(CH₃)₂), 2.27 (s, 6H, N(CH₃)₂), 1.68 (s, 6H, CH₃)

13C NMR (100 MHz, CDCl3), δ: 168.5 (C(O)CH=C), 161.0 (C=O), 106.7 (C(CH₃)₂), 94.6 (C(O)CH), 60.7 (CH₂N(CH₃)₂), 45.5 (N(CH₃)₂), 25.0 (CH₃)

FTIR (neat), cm⁻¹: 2947 (w), 2778 (w), 1731 (s, C=O), 1637 (m), 1392 (m), 1275 (m), 1204 (m), 1012 (m), 902 (w), 811 (m)

Found: 185.1051

TLC (80% EtOAc in hexanes), Rf: 0.12
n-Butyllithium (220 µL of a 1.25 M solution in hexanes, 277 µmol, 2.20 equiv) was added to a degassed, cooled (−78 °C) solution of diisopropylamine (40 µL, 283 µmol, 2.25 equiv) in tetrahydrofuran (1.0 mL). The resulting solution was warmed to 0 °C for 10 min and was then cooled to −78 °C. A solution of amine 110 (48.9 mg, 264 µmol, 2.1 equiv) in THF (500 µL) was added to the reaction mixture. After 35 min, benzaldehyde (13 µL, 126 µmol, 1 equiv) was added via syringe. After 20 min, the reaction mixture was quenched with water (5 mL) and was warmed to 23 °C. The product was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (70–80% ethyl acetate–hexanes) provided the aldol adduct 113 (26.3 mg, 72%) as a colorless oil. The $^{13}$C NMR spectral assignments were confirmed by an APT experiment.

$^1$H NMR (300 MHz, CDCl$_3$), δ:

7.40 (d, 2H, $J = 7.6$ Hz, o-aryl H), 7.32 (t, 2H, $J = 7.4$ Hz, m-aryl H), 7.23 (d, 1H, $J = 7.2$ Hz, p-aryl H), 6.02 (s, 1H, CHOH), 2.70 (ABq, 2H, $J = 13.3$ Hz, $\Delta \nu = 155.8$ Hz, CH$_2$N(CH$_3$)$_2$), 2.21 (s, 6H, N(CH$_3$)$_2$), 1.72 (s, 3H, CH$_3$), 1.67 (s, 3H, CH$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ:

165.1 (C(O)C=C), 161.8 (C=O), 143.8, 128.1 (2 aryl CH), 126.8, 125.2 (2 aryl CH), 113.3 (C(O)C), 105.7 (C(CH$_3$)$_2$), 67.0 (CHOH), 59.5 (CH$_2$N(CH$_3$)$_2$), 44.8 (N(CH$_3$)$_2$), 25.4 (CH$_3$),
FTIR (neat), cm\(^{-1}\):

- 3422 (w, OH)
- 2940 (w)
- 1726 (s, C=O)
- 1623 (m)
- 1391 (m)
- 1273 (m)
- 1202 (m)
- 1016 (m)
- 701 (m)

HRMS (Cl):

Calc'd for C\(_{16}\)H\(_{22}\)N\(_4\) [M + H]\(^+\): 292.1549

Found: 292.1542

TLC (EtOAc), \(R_f\):

0.40
n-Butyllithium (123 µL of a 1.48 M solution in hexanes, 182 µmol, 1.30 equiv) was added to a degassed, cooled (-78 °C) solution of diisopropylamine (26 µL, 189 µmol, 1.35 equiv) in tetrahydrofuran (1.0 mL). The resulting solution was warmed to 0 °C for 10 min and was then cooled to -78 °C. A solution of amine 110 (31.1 mg, 168 µmol, 1.2 equiv) in THF (500 µL) was added to the reaction mixture. After 60 min, a solution of benzoyl cyanide (18 mg, 140 µmol, 1 equiv) in tetrahydrofuran (500 µL) was added. After an additional 20 min, the reaction mixture was quenched upon addition of water (5 mL) and was warmed to 23 °C. The product was extracted with ethyl acetate (3 x 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (40% ethyl acetate–hexanes) gave the aldol adduct 114 (7.6 mg, 19%).

\[ \text{LDA, THF, } -78 \degree C \]

\[ \text{PhCOCN, } -78 \degree C \]

19%
FTIR (neat), cm$^{-1}$: 2945 (w), 1730 (s, C=O), 1627 (m), 1391 (m), 1272 (m), 1204 (m), 1015 (m), 693 (m)

HRMS (CI): Calc'd for C$_{16}$H$_{20}$NO$_4$ [M + H]$^+$: 290.1392

Found: 290.1390

TLC (EtOAc), $R_f$: 0.70
Trifluoroacetic acid (66 µL, 852 µmol, 2.5 equiv) was added to an ice-cooled solution of enone 96 (86.7 mg, 341 µmol, 1 equiv) in a 1:1 mixture of tetrahydrofuran in water (4.0 mL). After 2.3 h, the reaction mixture was diluted with water (4 mL) and the product was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography of the residue (60% ethyl acetate–hexanes) provided the deprotected enone 115 (38.0 mg, 79%) as a volatile, colorless oil.

$^{1}$H NMR (300 MHz, CDCl$_3$), δ:

- 6.59 (d(quintet), 1H, $J = 10.4$, 2.4 Hz, C(O)CH=CH), 6.02 (dt, 1H, $J = 10.5$, 1.9 Hz, C(O)CH), 3.98 (s, 2H, CH$_2$OH), 3.80 (br s, 1H, epoxide H), 3.00 (dd, 1H, $J = 21.4$, 4.7 Hz, CH$_2$), 2.75 (dq, 1H, $J = 21.4$, 2.6 Hz, CH$_2$), 2.35 (br, 1H, CH$_2$OH)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ:

- 195.8 (C=O), 143.7 (C(O)CH=CH), 126.9 (C(O)CH), 60.1, 59.8 (quaternary epoxide C), 56.6, 27.5

FTIR (neat), cm$^{-1}$:

- 3418 (br, OH), 2932 (w), 1669 (s, C=O), 1404 (m), 1057 (m), 847 (m)

HRMS (Cl):

Calc'd for C$_7$H$_9$O$_3$ [M + H]$^+$: 141.0552

Found: 141.0552

TLC (50% EtOAc in hexanes), $R_f$: 0.15
Methoxylamine hydrochloride (2.26 g, 27.0 mmol, 2.0 equiv) and pyridine (3.30 mL, 40.5 mmol, 3.0 equiv) were added sequentially to an ice-chilled solution of epoxy alcohol 94 (1.92 g, 13.5 mmol, 1 equiv) in methanol (30 mL). The resulting solution was stirred at 23 °C for 5 h. The reaction mixture was poured into water (100 mL), and the product was extracted with ethyl acetate (3 × 80 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (20–30% ethyl acetate–hexanes) provided methyloxime 116 (1.95 g) in 84% yield.

$^1$H NMR (300 MHz, CDCl$_3$), δ:
3.96 (br d, 1H, $J = 12.5$ Hz, CH$_2$OH), 3.86 (s, 3H, OCH$_3$), 3.73 (dd, 1H, $J = 12.5$, 8.0 Hz, CH$_2$OH), 3.43 (d, 1H, $J = 2.6$ Hz, epoxide H), 2.75 (m, 1H, CH$_2$OH), 2.64 (dt, 1H, $J = 18.4$, 4.0 Hz, N=CCH$_2$), 2.13 (dq, 1H, $J = 14.2$, 3.0 Hz, CH$_2$), 1.94 (m, 1H, CH$_2$), 1.42–1.79 (complex, 3H, CH$_2$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ:
155.2 (C=N), 63.2, 62.2, 58.4, 57.5, 23.4, 22.2, 15.1

FTIR (neat), cm$^{-1}$:
3432 (br, OH), 2940 (m), 1436 (w), 1101 (w), 1049 (s), 902 (w), 763 (w)

HRMS (EI):
Calc'd for C$_8$H$_{12}$NO$_3$ [M – H]$^+$: 170.0817
Found: 170.0823

TLC (40% EtOAc in hexanes), $R_f$: 0.41
n-Butyllithium (3.20 mL of a 2.44 M solution in hexanes, 7.79 mmol, 2.2 equiv) was added dropwise via syringe over 5 min to a degassed, chilled (-78 °C) solution of methyloxime 116 (606 mg, 3.54 mmol, 1 equiv) in tetrahydrofuran (10.0 mL). The reaction mixture was maintained at -78 °C for 2.5 h. A solution of phenylselenenyl chloride (817 mg, 4.27 mmol, 1.2 equiv) in THF (3.0 mL) was added to the reaction mixture. After 45 min, the reaction mixture was quenched with addition of water (15 mL), diluted with ethyl acetate (10 mL), and was warmed to 23 °C. The layers were separated and the aqueous layer was further extracted with ethyl acetate (2 × 10 mL). The combined organic layers were dried over sodium sulfate and were concentrated.

The resulting crude oil was cooled in a dichloromethane solution (20.0 mL) to -78 °C. m-Chloroperoxybenzoic acid (1.31 g, 5.31 mmol, 1.5 equiv) was added, and the resulting slurry was stirred at -78 °C for 17 min and at 0 °C for 17 min. The reaction mixture was quenched upon addition of a 1:4 mixture of saturated aqueous sodium carbonate solution and water (40 mL), was diluted with ethyl acetate (40 mL), and was warmed to 23 °C. The layers were separated, and the aqueous layer was further extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The crude oil was purified by flash chromatography (20–30% ethyl acetate–hexanes) to afford the α,β-unsaturated methyloxime 117 (356 mg, 59%) and the starting material 116 (71.1 mg, 12%). The $^{13}$C NMR spectral assignments were confirmed by an APT experiment.
$^1$H NMR (300 MHz, CDCl$_3$), $\delta$: 6.57 (dt, 1H, $J = 10.4$, 2.2 Hz, N=CCH=CH), 5.92 (dm, 1H, $J = 10.6$ Hz, N=CCH), 4.06 (d, 1H, $J = 12.7$ Hz, CH$_2$OH), 3.91 (s, 3H, OCH$_3$), 3.84 (br d, 1H, $J = 12.7$ Hz, CH$_2$OH), 3.56 (m, 1H, epoxide H), 2.81 (ddt, 1H, $J = 21.0$, 4.9, 1.4 Hz, CH$_2$), 2.70 (br, 1H, CH$_2$OH), 2.60 (dq, 1H, $J = 21.0$, 2.8 Hz, CH$_2$)

$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$: 149.9 (C=N), 132.5 (N=CCH=CH), 115.0 (N=CCH), 62.4 (OCH$_3$), 62.3 (CH$_2$OH), 58.2 (epoxide C), 55.5 (epoxide CH), 27.0 (CH$_2$)

FTIR (neat), cm$^{-1}$: 3419 (br, OH), 2939 (w), 1634 (w, C=N), 1411 (w), 1052 (s), 903 (m), 746 (w)

HRMS (EI): Calc'd for C$_8$H$_{10}$NO$_3$ [M - H]$^+$: 168.0661

Found: 168.0664

TLC (50% EtOAc in hexanes), $R_f$: 0.39
The Dess–Martin periodinane (380 mg, 895 µmol, 1.5 equiv) was added to an ice-cooled solution of alcohol 117 (101 mg, 597 µmol, 1 equiv) in dichloromethane (3.0 mL). The resulting slurry was stirred vigorously at 23 °C for 25 min. The reaction mixture was diluted with diethyl ether (6 mL) and a 1:1 mixture of saturated aqueous sodium bicarbonate solution and saturated aqueous sodium thiosulfate solution (6 mL) and was stirred at 23 °C. After 35 min, the layers were separated, and the aqueous layer was further extracted with diethyl ether (2 × 6 mL). The combined organic layers were dried over sodium sulfate and were concentrated to give the crude aldehyde 118 (88.5 mg, ~89%) as a yellow solid.

\[ \text{H NMR (300 MHz, CDCl}_3\text{), } \delta: \]
10.07 (s, 1H, CHO), 6.66 (dt, 1H, J = 10.6, 2.2 Hz, N=CCH=CH), 5.95 (dm, 1H, J = 10.6 Hz, N=CCH), 3.98 (s, 3H, OCH\textsubscript{3}), 3.62 (br, 1H, epoxide H), 2.86 (dd, 1H, J = 21.1, 4.4 Hz, CH\textsubscript{2}), 2.69 (dq, 1H, J = 21.0, 2.9 Hz, CH\textsubscript{2})

\[ \text{FTIR (neat), cm}^{-1}: \]
3424 (br), 2940 (w), 1729 (C=O), 1630 (w), 1412 (w), 1051 (s), 909 (m), 830 (m)

\[ \text{TLC (60% EtOAc in hexanes), } R_f: \]
0.43–0.66
The Dess–Martin periodinane (368 mg, 868 µmol, 1.5 equiv) was added to an ice-cooled solution of alcohol 116 (99.1 mg, 579 µmol, 1 equiv) in dichloromethane (3.0 mL). The resulting slurry was stirred vigorously at 23 °C for 20 min. The reaction mixture was diluted with diethyl ether (5 mL) and a 1:1 mixture of saturated aqueous sodium bicarbonate solution and saturated aqueous sodium thiosulfate solution (5 mL) and was stirred at 23 °C. After 30 min, the layers were separated, and the aqueous layer was further extracted with diethyl ether (2 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated to give the crude aldehyde 119 (89.9 mg, ~92%) as a yellow solid.

$^1$H NMR (300 MHz, CDCl$_3$), δ: 9.94 (s, 1H, CHO), 3.93 (s, 3H, OCH$_3$), 3.54 (d, 1H, $J$ = 2.6 Hz, epoxide H), 2.83 (dt, 1H, $J$ = 17.9, 3.1 Hz, CH$_2$), 2.23 (dq, 1H, $J$ = 15.2, 3.2 Hz, CH$_2$), 1.52–1.96 (complex, 4H, CH$_2$)

TLC (50% EtOAc in hexanes), $R_f$: 0.32–0.62
$n$-Butyllithium (285 µL of a 1.25 M solution in hexanes, 358 µmol, 2.20 equiv) was added to a degassed, chilled (-78 °C) solution of diisopropylamine (51 µL, 366 µmol, 2.25 equiv) in tetrahydrofuran (1.0 mL). The solution was warmed to 0 °C for 10 min and was then cooled to -78 °C. A solution of amine 110 (64.1 mg, 346 µmol, 2.1 equiv) in THF (500 µL) was added to the reaction mixture. The resulting solution was maintained at -78 °C for 35 min and was then added to the crude aldehyde 119 (27.5 mg, 163 µmol, 1 equiv) at -78 °C. After 25 min, water (5 mL) was added to the reaction mixture, and the solution was warmed to 23 °C. The product was extracted with ethyl acetate (3 x 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (50–100% ethyl acetate–hexanes) provided the aldol adduct 120 (10.1 mg, 18%) and its diastereomer 121 (3.9 mg, 7%) in addition to aldehyde 119 (12.9 mg, 47%). The diastereomers were arbitrarily assigned. The $^{13}$C NMR spectral assignments were confirmed by an APT experiment.

Aldol adduct 120:

$^1$H NMR (300 MHz, CDCl$_3$), δ: 7.86 (br, 1H, OH), 5.35 (s, 1H, CHOH), 4.30 (d, 1H, $J = 13.7$ Hz, CH$_2$N(CH$_3$)$_2$), 3.93 (br, 1H, epoxide H), 3.82 (s, 3H, OCH$_3$), 2.56 (dt, 1H, $J = 18.1, 4.2$ Hz, CH$_2$), 2.52 (d, 1H, $J = 13.9$ Hz, CH$_2$N(CH$_3$)$_2$), 2.28 (s, 6H, N(CH$_3$)$_2$), 1.96–2.17 (complex, 2H, CH$_2$), 1.46–1.82 (complex, 3H, CH$_2$), 1.70 (s, 3H, CH$_3$), 1.68 (s,
$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$:  

163.8 (C(O)C=O), 162.1 (C=O), 154.3 (C=N), 111.8 (C(O)C), 104.8 (C(CH$_3$)$_2$), 63.4 (OCH$_3$), epoxide C), 61.9 (epoxide CH), 60.6 (CH$_2$N(CH$_3$)$_2$), 55.6 (CHOH), 44.6 (N(CH$_3$)$_2$), 27.3 (CH$_3$), 24.1 (CH$_3$), 23.3 (CH$_2$), 22.4 (CH$_2$), 15.5 (CH$_2$)

FTIR (neat), cm$^{-1}$:  
3434 (br, OH), 2943 (m), 1729 (s, C=O), 1627 (m), 1395 (m), 1275 (m), 1206 (m), 1045 (s)

HRMS (Cl):  
Calc'd for C$_{17}$H$_{27}$N$_2$O$_6$ [M + H]$^+$: 355.1869
Found: 355.1873

TLC (EtOAc), $R_f$:  
0.37

Aldol adduct 121:

$^1$H NMR (300 MHz, CDCl$_3$), $\delta$:  
5.33 (s, 1H, CHOH), 3.79 (s, 3H, OCH$_3$), 3.76 (br, 1H, epoxide H), 3.33 (ABq, 2H, $J = 14.0$ Hz, $\Delta v = 150.5$ Hz, CH$_2$N(CH$_3$)$_2$), 2.80 (dt, 1H, $J = 17.4$, 3.4 Hz, CH$_2$), 2.35 (s, 6H, N(CH$_3$)$_2$), 2.16 (dq, 1H, $J = 15.0$, 3.4 Hz, CH$_2$), 1.49–2.00 (complex, 4H, CH$_2$), 1.69 (s, 3H, CH$_3$), 1.66 (s, 3H, CH$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$:  
167.2 (C(O)C=O), 160.6 (C=O), 155.2 (C=N), 108.0 (C(O)C), 105.5 (C(CH$_3$)$_2$), 64.1 (OCH$_3$), 61.7 (epoxide CH), 59.6 (epoxide C), 59.2 (CH$_2$N(CH$_3$)$_2$), 57.1 (CHOH), 45.5 (N(CH$_3$)$_2$), 26.6 (CH$_3$), 23.4 (CH$_3$), 23.0 (CH$_2$), 21.9 (CH$_2$), 15.4 (CH$_2$)
FTIR (neat), cm⁻¹:
3415 (br, OH), 2942 (m), 1728 (s, C=O), 1627 (m), 1391 (m), 1271 (m), 1206 (m), 1046 (s)

HRMS (FAB):
Calc'd for C_{17}H_{27}N_{2}O_{6} [M + H]^+: 355.1869
Found: 355.1880

TLC (EtOAc), R_f:
0.15
n-Butyllithium (215 µL of a 2.35 M solution in hexanes, 505 µmol, 2.2 equiv) was added to a degassed, chilled (−78 °C) solution of diisopropylamine (74 µL, 528 µmol, 2.3 equiv) in tetrahydrofuran (1.5 mL). The solution was warmed to 0 °C for 10 min and was then cooled to −78 °C. A solution of amine 110 (90.1 mg, 486 µmol, 2.1 equiv) in THF (1.0 mL) was added to the reaction mixture. The resulting solution was maintained at −78 °C for 35 min, at which point a solution of the crude aldehyde 118 (38.4 mg, 230 µmol, 1 equiv) in tetrahydrofuran (500 µL) was added. After 30 min, water (5 mL) was added to the reaction mixture, and the solution was warmed to 23 °C. The product was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (60% ethyl acetate–hexanes) provided the aldol adduct 122 (5.5 mg, 7%), based on its similarity to aldol adduct 120. The diastereomer of 122 was visible by TLC ($R_f = 0.10$ in ethyl acetate) but was not isolable.

$^1$H NMR (300 MHz, CDCl$_3$), δ:

6.56 (dt, 1H, J = 10.6, 1.9 Hz, N=CCH=CH), 5.92 (dm, 1H, J = 10.5 Hz, N=CCH), 5.44 (s, 1H, CHOH), 4.35 (d, 1H, J = 13.9 Hz, CH$_2$N(CH$_3$)$_2$), 4.08 (br, 1H, epoxide H), 3.87 (s, 3H, OCH$_3$), 2.81 (m, 1H, CH$_2$), 2.64 (dq, 1H, J = 21.0, 2.7 Hz, CH$_2$), 2.50 (d, 1H, J = 13.9 Hz, CH$_2$N(CH$_3$)$_2$), 2.29 (s, 6H, N(CH$_3$)$_2$), 1.70 (s, 3H, CH$_3$), 1.69 (s, 3H, CH$_3$)
$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$: 163.9 (C(O)C=C), 161.9 (C=O), 149.5 (C=N), 131.8 (N=CCH=CH), 115.5 (N=CCH), 111.8 (C(O)C), 104.8 (C(CH$_3$)$_2$), 63.9, 62.8, 62.2, 60.8, 54.0, 44.5 (N(CH$_3$)$_2$), 27.4, 27.1, 24.0

FTIR (neat), cm$^{-1}$: 3418 (br, OH), 2940 (w), 1728 (s, C=O), 1627 (m), 1395 (m), 1274 (m), 1207 (m), 1047 (s)

HRMS (Cl): Calc'd for C$_{17}$H$_{25}$N$_2$O$_6$ [M+H]$^+$: 353.1712

Found: 353.1709

TLC (EtOAc), $R_f$: 0.34
Potassium bis(trimethylsilyl)amide (360 µL of a 0.5 M solution in toluene, 181 µmol, 1.7 equiv) was added to a degassed, chilled (−78 °C) solution of amine 110 (29.2 mg, 158 µmol, 1.5 equiv) in tetrahydrofuran (1.0 mL). After 1 h, a solution of crude aldehyde 118 (17.8 mg, 106 µmol, 1 equiv) in THF (500 µL) was added to the reaction mixture. After 20 min, the reaction mixture was quenched upon addition of water (5 mL), and the product was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The residue was purified by flash chromatography (60–70% ethyl acetate–hexanes) to provide the aldol adduct 123 (6.8 mg, 18%). The $^{13}$C NMR spectral assignments were confirmed by an APT experiment.

$^1$H NMR (300 MHz, CDCl$_3$), δ:

6.61 (dt, 1H, $J = 10.6$, 2.0 Hz, N=CCH=CH), 5.93 (dm, 1H, $J = 10.5$ Hz, N=CCH), 5.28 (s, 1H, C(O)CH), 4.89 (d, 1H, $J = 5.6$ Hz, CHN(CH$_3$)$_2$), 3.95 (s, 3H, OCH$_3$), 3.77 (br, 1H, epoxide H), 3.22 (d, 1H, $J = 5.6$ Hz, CHOH), 2.80 (dd, 1H, $J = 21.8$, 4.6 Hz, CH$_2$), 2.64 (br, 1H, OH), 2.54 (dq, 1H, $J = 21.4$, 2.8 Hz, CH$_2$), 2.36 (s, 6H, N(CH$_3$)$_2$), 1.73 (s, 3H, CH$_3$), 1.70 (s, 3H, CH$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ:

167.9, 160.7, 132.0 (N=CCH=CH), 114.7 (N=CCH), 110.0, 107.0, 96.3 (C(O)CH), 69.1
FTIR (neat), cm$^{-1}$: 3482 (br, OH), 2940 (w), 1729 (C=O), 1625 (m), 1392 (m), 1272 (m), 1203 (m), 1050 (s)

HRMS (CI): Calc'd for C$_{17}$H$_{25}$N$_2$O$_6$ [M + H]$^+$: 353.1712
Found: 353.1706

TLC (EtOAc), $R_f$: 0.42
Sodium chlorite (414 mg of technical grade (80%) reagent, 3.66 mmol, 4.0 equiv) was added to a solution of crude aldehyde 118 (153 mg, 915 µmol, 1 equiv), 2-methyl-2-butene (2.30 mL of a 2.0 M solution in THF, 4.58 mmol, 5.0 equiv), and sodium dihydrogenphosphate (1.26 mL of a 1.45 M aqueous solution, 1.83 mmol, 2.0 equiv) in tert-butyl alcohol (4.5 mL; 0.20 M 118 in t-BuOH). The biphasic reaction mixture was stirred vigorously at 23 °C for 40 min. A 1:4 mixture of 1 N aqueous HCl solution and 0.9 M aqueous potassium dihydrogenphosphate solution (10 mL) and brine (2 mL) were added to the reaction mixture. The product was extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The crude oil was diluted with benzene (4.0 mL) and methanol (1.0 mL) at 23 °C. (Trimethylsilyl)diazomethane (825 µL of a 2.0 M solution in hexanes, 1.65 mmol, 1.8 equiv) was added to the reaction mixture. After 20 min, the reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography (20% ethyl acetate–hexanes) afforded methyl ester 130 (126 mg, 70%) as a pale yellow oil.

$^1$H NMR (300 MHz, CDCl$_3$), δ: 6.62 (dt, 1H, $J = 10.8$, 2.2 Hz, N=CCH=CH), 5.91 (dm, 1H, $J = 10.9$ Hz, N=CCH), 3.93 (s, 3H, NOCH$_3$), 3.82 (s, 3H, OCH$_3$), 3.64 (m, 1H, epoxide H), 2.83 (dm, 1H, $J = 21.6$ Hz, CH$_2$), 2.69 (dq, 1H, $J = 21.2$, 2.9 Hz, CH$_2$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ: 167.3 (C=O), 146.2 (C=N), 131.1
(N=CCH=CH), 114.5 (N=CCH), 94.7 (epoxide C), 62.6, 57.4 (epoxide CH), 56.5, 52.5, 26.4
(CH₂)

FTIR (neat), cm⁻¹:
2943 (w), 1749 (s, C=O), 1631 (w), 1441 (m),
1290 (m), 1248 (s), 1051 (s), 907 (m)

HRMS (Cl):
Calc'd for C₉H₁₂NO₄ [M – H]^+: 198.0766
Found: 198.0768

TLC (60% EtOAc in hexanes), Rf: 0.57
Triethylamine (270 µL, 1.93 mmol, 10.0 equiv) was added to a solution of methyl ester 130 (38.1 mg, 193 µmol, 1 equiv) in dichloromethane (2.0 mL). The reaction mixture was heated to reflux for 6 h. The reaction mixture containing the intermediate diene was then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (75 µL, 386 µmol, 2.0 equiv) was added dropwise by syringe. After 20 min, the reaction mixture was quenched upon the addition of water (5 mL) and brine (2 mL), diluted with ethyl acetate (5 mL), and was warmed to 23 °C. The layers were separated, and the aqueous layer was further extracted with ethyl acetate (2 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (10% ethyl acetate–hexanes) provided the trimethylsilyl ether 131 (44.4 mg, 85%) as a white solid.

\[ 130 \]  

1H NMR (300 MHz, CDCl₃), δ: 6.75 (d, 1H, \( J = 10.0 \) Hz, \( \text{CH} = \text{CH} \)), 6.16–6.30 (complex, 2H, \( \text{CH} = \text{CHCH} = \text{CH} \)), 5.96 (d, 1H, \( J = 9.5 \) Hz, \( \text{N} = \text{CCH} \)), 3.97 (s, 3H, NOCH₃), 3.74 (s, 3H, OCH₃), 0.13 (s, 9H, Si(CH₃)₃)

\[ 131 \]  

13C NMR (100 MHz, CDCl₃), δ: 171.5 (C=O), 153.7 (C=N), 131.9, 127.9, 123.7, 113.8, 74.3 (COTMS), 62.6 (NOCH₃), 52.8 (OCH₃), 1.9 (Si(CH₃)₃)

FTIR (neat), cm⁻¹: 2956 (w), 1756 (s, C=O), 1411 (w), 1250 (m), 1132 (s), 1051 (s), 842 (s)

HRMS (Cl): Calc'd for C₁₉H₁₉NO₄Si [M⁺]: 269.1083
Found: 269.1077
TLC (3:1:1 EtOAc:toluene:hexane),

\[ R_f: 0.58 \]
2-Carbethoxy-2-cyclohexen-1-one was prepared from 2-carbethoxycyclohexanone (1.06 g, 6.25 mmol, 1 equiv) by the procedure of Reich and co-workers. The crude product (th. yield = 6.25 mmol) was diluted with ethanol (10.0 mL) and was cooled to 0 °C. tert-Butyl hydroperoxide (2.50 mL of a 70% aqueous solution, 18.8 mmol, 3.0 equiv) and sodium hydroxide (105 µL of a 6 N aqueous solution, 625 µmol, 0.1 equiv) were added sequentially to the reaction mixture. After 30 min, the reaction mixture was poured into water (30 mL) and brine (10 mL), and the product was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (20% ethyl acetate–hexanes) afforded epoxide 132 (743 mg) in 65% yield from commercially available starting material.

**^1H NMR (300 MHz, CDCl₃), δ:**

4.27 (q, 2H, J = 7.1 Hz, OCH₂CH₃), 3.66 (m, 1H, epoxide H), 2.55 (dt, 1H, J = 17.2, 4.2 Hz, C(O)CH₂), 2.32 (m, 1H, C(O)CH₂), 1.71–2.21 (complex, 4H, CH₂), 1.29 (t, 3H, J = 7.2 Hz, OCH₂CH₃)

**^13C NMR (100 MHz, CDCl₃), δ:**

199.1 (C=O), 165.5 (CO₂Et), 61.3, 59.4, 58.1, 36.8, 22.2, 15.9, 13.5

**FTIR (neat), cm⁻¹:**

2956 (w), 1748 (s, C=O), 1717 (s, C=O), 1282 (m), 1224 (m), 1060 (m)

**HRMS (Cl):**

Calc'd for C₉H₁₃O₄ [M + H]⁺: 185.0814
Found: 185.0817

TLC (40% EtOAc in hexanes), $R_f$: 0.45
1. LDA, TMSCl, THF, -78 °C
2. PhSeCl, CH₂Cl₂, 0 °C
3. H₂O₂, CH₂Cl₂:H₂O, 23 °C

29%

**132**

**133**

*n*-Butyllithium (525 µL of a 2.35 M solution in hexanes, 1.24 mmol, 1.20 equiv) was added to a degassed, cooled (-78 °C) solution of diisopropylamine (180 µL, 1.29 mmol, 1.25 equiv) in tetrahydrofuran (4.0 mL). The resulting solution was stirred at 0 °C for 10 min and was then cooled to -78 °C. Chlorotrimethylsilane (325 µL, 2.58 mmol, 2.5 equiv) was added to the reaction mixture, followed 5 min later by the dropwise addition of ketone **132** (190 mg, 1.03 mmol, 1 equiv) in THF (1.0 mL). After 20 min, the reaction mixture was quenched upon addition of triethylamine (430 µL, 3.09 mmol, 3.0 equiv), and the resulting solution was poured into saturated aqueous sodium bicarbonate solution (10 mL). The product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 x 10 mL). The combined organic layers were dried over sodium sulfate and were concentrated.

The crude oil was diluted with dichloromethane (4.0 mL) and was cooled to 0 °C. A solution of phenylselenenyl chloride (237 mg, 1.24 mmol, 1.2 equiv) in dichloromethane (1.0 mL) was added, and the resulting reaction mixture was stirred at 0 °C. After 30 min, a 1:4 mixture of saturated aqueous sodium carbonate solution and water (10 mL) was added to the reaction mixture. The product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 x 8 mL). The combined organic layers were dried over sodium sulfate and were concentrated.

This crude oil was diluted with dichloromethane (4.0 mL) and water (1.0 mL) and was cooled to 0 °C. Hydrogen peroxide (257 mg of a 30% (w/w) aqueous solution, 2.27 mmol, 2.2 equiv) was added, and the reaction mixture was stirred vigorously at 23 °C. After 20 min, the reaction mixture was diluted with water (10 mL) and the product was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over
sodium sulfate and were concentrated. Rapid purification of the residue by flash chromatography (35% ethyl acetate–hexanes) provided enone 133 (53.7 mg, 29%).

$^1$H NMR (300 MHz, CDCl$_3$), δ:

- 6.58 (dm, 1H, $J = 10.6$ Hz, C(O)CH=CH)
- 6.02 (dt, 1H, $J = 10.5$, 2.2 Hz, C(O)CH)
- 4.26 (q, 2H, $J = 7.1$ Hz, OCH$_2$CH$_3$)
- 3.80 (m, 1H, epoxide H)
- 2.97 (ddm, 1H, $J = 22.3$, 4.8 Hz, CH$_2$)
- 2.81 (dq, 1H, $J = 21.6$, 2.8 Hz, CH$_2$)
- 1.28 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ:

- 188.7 (C=O)
- 165.4 (CO$_2$Et)
- 143.3 (C(O)CH=CH)
- 126.4 (C(O)CH)
- 62.0
- 58.1
- 57.7
- 26.5
- 13.9

FTIR (neat), cm$^{-1}$:

- 2986 (w)
- 1748 (s, C=O)
- 1680 (s, C=O)
- 1399 (m)
- 1291 (m)
- 1240 (m)
- 1063 (s)
- 824 (m)

HRMS (EI):

- Calc'd for C$_9$H$_{10}$O$_4$ [M]: 182.0579
- Found: 182.0580

TLC (30% EtOAc in hexanes), $R_f$: 0.15
Triethylamine (60 µL, 430 µmol, 10.0 equiv) was added to a solution of enone 133 (7.9 mg, 43 µmol, 1 equiv) in tetrahydrofuran (1.0 mL; 0.043 M 133 in THF) at 23 °C. The reaction mixture was warmed at 50 °C for 30 min and was then cooled to 23 °C. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography (25% ethyl acetate–hexanes; 50% ethyl acetate–hexanes) to provide lactone 135 (1.5 mg, 19%) as a colorless oil and dimer 136 (2.8 mg, 18%) as a yellow solid.

Lactone 135:

\( \text{Lactone 135:} \)

\( ^1\text{H NMR} \ (300 \text{ MHz, CDCl}_3), \delta: \)

\[ \begin{align*}
7.17 \,(d, \, 1\text{H}, \, J = 5.4 \text{ Hz}, \, \text{CH} = \text{CCO}_2\text{Et}), \\
6.39 \,(dd, \, 1\text{H}, \, J = 9.4, \, 5.7 \text{ Hz}, \, \text{CH} = \text{CHCH} = \text{C}), \\
6.07 \,(dt, \, 1\text{H}, \, J = 9.5, \, 6.4 \text{ Hz}, \, \text{CH}_2\text{CH}), \\
4.33 \,(q, \, 2\text{H}, \, J = 7.1 \text{ Hz}, \, \text{OCH}_2\text{CH}_3), \\
3.12 \,(d, \, 2\text{H}, \, J = 6.7 \text{ Hz}, \, \text{CH}_2), \\
1.36 \,(t, \, 3\text{H}, \, J = 7.2 \text{ Hz}, \, \text{OCH}_2\text{CH}_3)
\end{align*} \]

\( ^{13}\text{C NMR} \ (100 \text{ MHz, acetone-}d_6) \delta: \)

\[ \begin{align*}
166.1 \,(\text{C}=\text{O}), \\
161.9 \,(\text{C}=\text{O}), \\
141.6 \,(\text{C}C\text{O}_2\text{Et}), \\
128.4, \\
127.3, \\
120.0, \\
62.3, \\
36.7, \\
14.4
\end{align*} \]

\( \text{FTIR (neat), cm}^{-1}: \)

\[ \begin{align*}
2984 \,(w), \\
1777 \,(s, \, \text{C}=\text{O}), \\
1729 \,(s, \, \text{C}=\text{O}), \\
1597 \,(w), \\
1277 \,(s), \\
1122 \,(s)
\end{align*} \]

\( \text{HRMS (Cl)}: \)

Calc'd for \( \text{C}_9\text{H}_{10}\text{O}_4 [\text{M}]^+: \ 182.0579 \)

Found: 182.0577

\( \text{TLC (60\% EtOAc in hexanes), } R_f: \)

0.69
Dimer 136:

$^1$H NMR (300 MHz, CDCl$_3$), δ:

6.61 (dd, 1H, $J = 10.2$, 4.4 Hz, H$_b$), 6.34 (td, 1H, $J = 7.3$, 1.0 Hz, H$_t$), 6.16 (dd, 1H, $J = 10.0$, 1.7 Hz, H$_a$), 5.92 (td, 1H, $J = 7.3$, 1.3 Hz, H$_e$), 4.51 (s, 1H, OH), 4.15 (m, 4H, OCH$_2$CH$_3$), 3.87 (dd, 1H, $J = 8.2$, 2.0 Hz, H$_h$), 3.79 (s, 1H, OH), 3.58 (m, 2H, H$_c$, H$_g$), 3.36 (m, 1H, H$_d$), 1.22 (m, 6H, OCH$_2$CH$_3$)

$^{13}$C NMR (100 MHz, CD$_3$CN), δ:

205.4, 195.0, 171.1, 170.3, 149.6, 134.4, 130.0, 128.5, 79.7, 77.8, 63.3, 62.9, 52.4, 42.9, 41.5, 37.7, 14.2, 14.1

FTIR (neat), cm$^{-1}$:

3444 (br, OH), 2924 (w), 1734 (s, C=O), 1696 (s, C=O), 1254 (m), 1214 (m), 1125 (m), 1020 (s)

HRMS (Cl):

Calc'd for C$_{18}$H$_{24}$NO$_8$ [M + NH$_4$]$^+$: 382.1502

Found: 382.1507

TLC (60% EtOAc in hexanes), $R_f$: 0.50
Potassium bis(trimethylsilyl)amide (480 µL of a 0.5 M solution in toluene, 241 µmol, 3.0 equiv) was added to a degassed, cooled (-78 °C) solution of amine 110 (41.6 mg, 225 µmol, 2.8 equiv) in tetrahydrofuran (0.5 mL). The resulting solution was maintained at -78 °C for 75 min. Lithium bis(trimethylsilyl)amide (96 µL of a 1.0 M solution in THF, 96 µmol, 1.2 equiv) was added to a degassed, cooled (-78 °C) solution of enone 96 (20.4 mg, 80.2 µmol, 1 equiv) and chlorotrimethylsilane (13 µL, 104 µmol, 1.3 equiv) in tetrahydrofuran (2.0 mL; 0.040 M 96 in THF). The reaction mixture containing the amine substrate was then immediately transferred to the reaction mixture containing the enone substrate. The resulting reaction mixture was stirred at -78 °C for 25 min and was then warmed to -40 °C. Over the next 60 min, the reaction mixture was allowed to warm slowly to -15 °C. The reaction mixture was quenched upon the addition of water (5 mL), and the products were extracted with ethyl acetate (3 x 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (10–15% ethyl acetate–hexanes) afforded adduct 142 (5.3 mg, 13%) as an inseparable mixture of diastereomers (2.5:1), in addition to 2-t-butyldimethylsilyloxy phenol 138 (5.5 mg, 30%) and 2,3-dihydroxybenzyl TBS-ether 139 (1.7 mg, 8%).

Adduct 142 (major diastereomer):

\[^1\text{H} \text{NMR} \ (300 \text{ MHz, CDCl}_3), \delta: \]
\[
6.28 \ (d, 1H, J = 9.8 \text{ Hz, CHCOTMS}), \ 5.67 \ (dd, 1H, J = 10.2, 2.3 \text{ Hz, CH=CHCOTMS}), \ 5.18 \ (s, 1H, \text{C(O)CH}), \ 3.56 \ (\text{ABq, 2H, } J = 9.4 \text{ Hz, } \Delta v =
\]

110

142
$^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$: 208.5 (C=O), 166.2 (C(O)CH=CH), 160.2 (CO$_2$R), 132.7 (CH=CH), 131.5 (CH=CH), 107.1 (C(CH$_3$)$_2$), 96.3 (C(O)CH), 78.8, 71.2, 69.9, 42.9, 41.3 (N(CH$_3$)$_2$), 36.4, 25.9 (SiC(CH$_3$)$_3$), 25.8 (CH$_3$), 25.5 (CH$_3$), 18.3 (SiC(CH$_3$)$_3$), 2.0 (Si(CH$_3$)$_3$), -5.4 (Si(CH$_3$)$_3$)

FTIR (neat), cm$^{-1}$: 2954 (m), 1734 (s, C=O), 1623 (w), 1389 (m), 1252 (m), 1121 (m), 841 (s)

HRMS (Cl): Calc'd for C$_{25}$H$_{46}$NO$_6$Si$_2$ [M + H]$^+$: 512.2863

Found: 512.2863

TLC (30% EtOAc in hexanes), $R_f$: 0.48
Triisopropylsilyl trifluoromethanesulfonate (245 µL, 913 µmol, 1.1 equiv) was added to an ice-chilled solution of 2-hydroxybenzyl alcohol (103 mg, 830 µmol, 1 equiv) and triethylamine (175 µL, 1.24 mmol, 1.5 equiv) in dichloromethane (5.0 mL). After 25 min, the reaction mixture was poured into water (50 mL), and the product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 × 50 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the crude residue by flash chromatography (10% ethyl acetate–hexanes) gave phenol 154 (215 mg, 92%) as a colorless oil.

$^1$H NMR (300 MHz, CDCl$_3$), δ: 8.27 (s, 1H, OH), 7.18 (t, 1H, $J = 7.6$ Hz, aryl H), 6.93 (d, 1H, $J = 7.2$ Hz, aryl H), 6.88 (d, 1H, $J = 8.0$ Hz, aryl H), 6.81 (t, 1H, $J = 7.4$ Hz, aryl H), 4.99 (s, 2H, CH$_2$), 1.18 (m, 3H, SiCH), 1.90 (d, 18H, Si(CH(CH$_3$)$_2$)$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ: 156.6, 128.8, 126.5, 124.1, 119.4, 116.6, 66.3, 17.8, 11.7

FTIR (neat), cm$^{-1}$: 3373 (br, OH), 2944 (s), 2867 (s), 1590 (m), 1463 (m), 1244 (m), 1052 (s), 882 (m), 753 (s)

HRMS (Cl): Calc'd for C$_{16}$H$_{29}$O$_2$Si [M + H]$^+$: 281.1937

Found: 281.1940

TLC (30% EtOAc in hexanes), $R_f$: 0.78
Lead(IV) acetate (49 mg, 111 µmol, 1.5 equiv) was added to a solution of phenol 154 (20.8 mg, 74.1 µmol, 1 equiv) in acetic acid (1.5 mL) at 23 °C. After 2 h, saturated aqueous sodium bicarbonate solution (5 mL) was added to the reaction mixture, and the product was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (5% ethyl acetate–hexanes) afforded the 2,4-cyclohexadienone 155 (6.7 mg, 27%) as a yellow oil.

$^1$H NMR (300 MHz, CDCl$_3$), δ: 7.00 (ddd, 1H, $J = 9.8$, 5.8, 1.9 Hz, C(O)CH=CH), 6.41 (ddd, 1H, $J = 9.7$, 5.7, 0.9 Hz, CH=CH), 6.33 (br d, 1H, $J = 9.8$ Hz, CH=CH), 6.17 (d, 1H, $J = 9.9$ Hz, C(O)CH), 3.90 (ABq, 2H, $J = 9.3$ Hz, $\Delta v = 19.9$ Hz, CH$_2$), 2.09 (s, 3H, CH$_3$), 1.02 (m, 21H, Si(CH(CH$_3$)$_2$)$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ: 197.7, 169.2, 140.7, 139.7, 126.9, 124.3, 80.9, 69.2, 20.4, 17.8, 11.8

FTIR (neat), cm$^{-1}$: 2944 (s), 2866 (s), 1749 (s, C=O), 1680 (s, C=O), 1464 (m), 1369 (m), 1242 (s), 1128 (s)

HRMS (CI): Calc'd for C$_{18}$H$_{34}$NO$_4$Si [M + NH$_4$]$^+$: 356.2257

Found: 356.2250

TLC (10% EtOAc in hexanes), $R_f$: 0.23
tert-Butyldimethylsilyl chloride (424 mg, 2.82 mmol, 2.0 equiv) was added to a solution of salicylaldehyde (172 mg, 1.41 mmol, 1 equiv) and triethylamine (590 µL, 4.22 mmol, 3.0 equiv) in tetrahydrofuran (8.0 mL) at 23 °C. 4-Dimethylaminopyridine (1–2 mg) was added to the reaction mixture. After 40 min, the reaction mixture was poured into water (50 mL), and the product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 × 50 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography of the residue (4% ethyl acetate–hexanes) provided the protected aldehyde 159 (334 mg, 100% yield) as a colorless oil.

$^1$H NMR (300 MHz, CDCl$_3$), δ: 10.47 (s, 1H, CHO), 7.81 (dd, 1H, $J = 7.8$, 1.8 Hz, C-6 aryl H), 7.46 (td, 1H, $J = 7.8$, 1.8 Hz, C-4 aryl H), 7.03 (t, 1H, $J = 7.6$ Hz, C-5 aryl H), 6.88 (d, 1H, $J = 8.3$ Hz, C-3 aryl H), 1.02 (s, 9H, SiC(CH$_3$)$_3$), 0.28 (s, 6H, Si(CH$_3$)$_2$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ: 190.1 (C=O), 158.9, 135.6, 128.3, 127.3, 121.4, 120.2, 25.7 (SiC(CH$_3$)$_3$), 18.3 (SiC(CH$_3$)$_3$), -4.3 (Si(CH$_3$)$_2$)

FTIR (neat), cm$^{-1}$: 2932 (m), 2859 (m), 1690 (s, C=O), 1599 (s), 1479 (s), 1253 (s), 916 (s)

HRMS (EI): Calc'd for C$_{13}$H$_{21}$O$_2$Si [$M + H]^+$: 237.1311

Found: 237.1305

TLC (10% EtOAc in hexanes), $R_f$: 0.47
n-Butyllithium (1.67 mL of a 2.52 M solution in hexanes, 4.20 mmol, 1.20 equiv) was added to a degassed, cooled (−78 °C) solution of diisopropylamine (615 µL, 4.37 mmol, 1.25 equiv) in tetrahydrofuran (12.0 mL). The resulting solution was warmed to 0 °C for 10 min and was then cooled to −78 °C. A solution of amine 110 (648 mg, 3.50 mmol, 1 equiv) in THF (2.0 mL) was added. After 50 min, aldehyde 159 (1.07 g, 4.53 mmol, 1.3 equiv) was added by syringe to the reaction mixture. After an additional 40 min, the reaction mixture was poured into water (60 mL), and the product was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (60% ethyl acetate–hexanes) afforded the aldol adduct 160 (1.12 g, 76%) as a white solid.

^1^H NMR (300 MHz, CDCl\textsubscript{3}), δ: 7.59 (d, 1H, J = 7.4 Hz, C-6 aryl H), 7.13 (t, 1H, J = 7.5 Hz, C-4 aryl H), 6.97 (t, 1H, J = 7.4 Hz, C-5 aryl H), 6.82 (d, 1H, J = 8.0 Hz, C-3 aryl H), 6.06 (s, 1H, CH\textsubscript{OH}), 2.70 (ABq, 2H, J = 13.6 Hz, Δν = 184.4 Hz, CH\textsubscript{2}N(CH\textsubscript{3})\textsubscript{2}), 2.25 (s, 6H, N(CH\textsubscript{3})\textsubscript{2}), 1.65 (s, 3H, CH\textsubscript{3}), 1.62 (s, 3H, CH\textsubscript{3}), 0.93 (s, 9H, SiC(CH\textsubscript{3})\textsubscript{3}), 0.31 (s, 3H, Si(CH\textsubscript{3})\textsubscript{2}), 0.16 (s, 3H, Si(CH\textsubscript{3})\textsubscript{2})

^1^3^C NMR (100 MHz, CDCl\textsubscript{3}), δ: 163.8 (C(O)C=), 161.4 (C=O), 152.5, 133.8, 127.8, 126.7, 120.3, 119.4, 113.0 (C(O)C), 105.1 (C(CH\textsubscript{3})\textsubscript{2}), 63.9 (CH\textsubscript{OH}), 59.1
FTIR (neat), cm⁻¹:
- 3190 (w, OH), 2954 (w), 2859 (w), 1731 (s, C=O), 1631 (m), 1483 (m), 1395 (m), 1271 (s), 1030 (m), 837 (m)

HRMS (EI):
- Calc'd for C₂₂H₃₆NO₅Si [M + H]⁺: 422.2363
- Found: 422.2365

TLC (80% EtOAc in hexanes), Rf: 0.39

(CH₂N(CH₃)₂), 44.7 (N(CH₃)₂), 26.9, 26.3, 23.5, 18.8, -3.0 (Si(CH₃)₂), -3.8 (Si(CH₃)₂)
Trifluoroacetic acid (26 µL, 339 µmol, 1.1 equiv) was added to an ice-chilled solution of alcohol 160 (130 mg, 308 µmol, 1 equiv) in dichloromethane (5.0 mL). After 5 min, Dess–Martin periodinane (196 mg, 463 µmol, 1.5 equiv) was added to the reaction mixture, and the resulting slurry was stirred vigorously at 23 °C. After 20 min, the reaction mixture was diluted with diethyl ether (7 mL) and a 1:1 mixture of saturated aqueous sodium bicarbonate solution and saturated aqueous sodium thiosulfate solution (10 mL) and was stirred at 23 °C. After 30 min, the layers were separated, and the aqueous layer was further extracted with diethyl ether (2 × 10 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (70–80% ethyl acetate–hexanes) provided ketone 161 (102 mg, 79%) as a yellow oil.

$^1$H NMR (300 MHz, CDCl$_3$), δ:

7.49 (dd, 1H, $J = 7.8$, 1.8 Hz, C-6 aryl H), 7.34 (td, 1H, $J = 7.8$, 1.8 Hz, C-4 aryl H), 6.97 (t, 1H, $J = 7.4$ Hz, C-5 aryl H), 6.85 (d, 1H, $J = 8.2$ Hz, C-3 aryl H), 3.26 (s, 2H, CH$_2$N(CH$_3$)$_2$), 2.26 (s, 6H, N(CH$_3$)$_2$), 1.78 (s, 6H, CH$_3$), 0.97 (s, 9H, SiC(CH$_3$)$_3$), 0.24 (s, 6H, Si(CH$_3$)$_2$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ:

189.8 (C=O), 170.5 (C(O)C=C), 158.5 (CO$_2$R), 154.6, 132.8, 131.2, 130.4, 120.6, 120.1, 111.7 (C(O)C), 106.6 (C(CH$_3$)$_2$), 58.4 (CH$_2$N(CH$_3$)$_2$), 45.4 (N(CH$_3$)$_2$), 25.9, 25.4, 18.5, -4.1
FTIR (neat), cm⁻¹:

2933 (w), 2858 (w), 1737 (s, C=O), 1598 (m), 1481 (m), 1389 (m), 1272 (s), 1205 (m)

HRMS (EI):

Calc’d for C₂₅H₃₂N₀₅Si [M – H]⁺: 418.2050

Found: 418.2047

TLC (EtOAc), Rᵢ:

0.34
Triethylamine trihydrofluoride (2 × 10 µL) was added in two portions to a solution of alcohol 160 (8.7 mg, 20.6 µmol, 1 equiv) in dichloromethane (1.0 mL) at 23°C. After the reaction was complete by TLC, the reaction mixture was diluted with water (5 mL) and 10% methanol in dichloromethane (4 mL). The layers were separated, and the aqueous layer was further extracted with 10% methanol in dichloromethane (2 × 3 mL). The combined organic layers were dried over sodium sulfate and were concentrated. The crude diol 162 was used immediately in subsequent reactions. Over time or under slightly acidic conditions, diol 162 underwent a rearrangement to amine 163.

Amine 163:

$^1$H NMR (300 MHz, CDCl$_3$), δ: 7.45 (s, 1H, C(O)C=CH), 7.30 (m, 2H, aryl H), 6.99 (m, 2H, aryl H), 2.58 (s, 2H, CH$_2$N(CH$_3$)$_2$), 2.28 (s, 6H, N(CH$_3$)$_2$), 1.72 (s, 3H, CH), 1.69 (s, 3H, CH$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$), δ: 163.4, 152.8, 132.6, 131.8, 129.3, 122.1, 119.9, 119.8, 116.6, 104.6, 101.2, 62.9, 46.9, 28.4, 26.9

FTIR (neat), cm$^{-1}$: 2945 (w), 2776 (w), 1739 (s, C=O), 1644 (m), 1273 (s), 1214 (m), 1123 (m), 1047 (m), 769 (m)

HRMS (CI): Calc'd for C$_{16}$H$_{20}$NO$_4$ [M + H]$^+$: 290.1392

Found: 290.1386

TLC (80% EtOAc in hexanes), $R_f$: 0.78
A buffered solution of hydrogen fluoride•pyridine in tetrahydrofuran was prepared by the addition of 70% hydrogen fluoride•pyridine (500 µL) to a solution of pyridine (2.0 mL) in THF (5.0 mL) at 0 °C. A 100-µL aliquot was added to a solution of ketone 161 (4.9 mg, 12 µmol, 1 equiv) in tetrahydrofuran (1.0 mL) at 23 °C. After 35 min, the reaction mixture was diluted with water (5 mL), and the product was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated to a volume of approximately 500 µL. The pyridine solution was then concentrated to dryness from benzene (2 × 2 mL) to afford the crude phenol 167 (1.8 mg, ~50%).

\[
\begin{align*}
\text{H NMR (300 MHz, C}_6\text{D}_6, \delta): & \quad 7.89 (dd, 1H, J = 7.8, 1.6 Hz, C-6 aryl H), 7.06 (t, 1H, J = 7.3 Hz, C-4 aryl H), 6.93 (d, 1H, J = 8.0 Hz, C-3 aryl H), 6.81 (t, 1H, J = 7.6 Hz, C-5 aryl H), 3.01 (br d, 1H, J = 12.9 Hz, CH}_2\text{N(CH}_3\text{)}_2, 2.50 (d, 1H, J = 13.2 Hz, CH}_2\text{N(CH}_3\text{)}_2, 2.19 (s, 6H, N(CH}_3\text{)}_2, 1.54 (s, 3H, CH}_3), 1.45 (s, 3H, CH}_3) \\
\text{FTIR (neat), cm}^{-1}: & \quad 3406 (br, OH), 2941 (w), 1682 (s, C=O), 1607 (m), 1427 (m), 1272 (m), 1189 (m), 761 (m) \\
\text{TLC (EtOAc), } R_f: & \quad 0.04
\end{align*}
\]
tert-Butyldimethylsilyl chloride (688 mg, 4.57 mmol, 1.4 equiv) was added to a solution of 4-hydroxy-2-methoxybenzaldehyde (497 mg, 3.27 mmol, 1 equiv) and triethylamine (685 µL, 4.90 mmol, 1.5 equiv) in tetrahydrofuran (13.0 mL) at 23 °C. After 50 min, the reaction mixture was poured into water (50 mL), and the product was extracted with a 1:1 mixture of ethyl acetate and hexanes (3 × 50 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Flash chromatography of the residue (5% ethyl acetate–hexanes) provided the protected aldehyde 172 (778 mg, 89%) as a white solid.

\[\begin{array}{c}
\text{HO} & \quad \text{TBSCI, Et}_3\text{N} & \quad \text{TBSO}^+ \text{CHO} \\
\text{H}_3\text{CO} & \quad \text{THF, 23}^\circ\text{C} & \quad 89\% \\
\end{array}\]

172

1H NMR (300 MHz, CDCl$_3$), δ:

- 10.29 (s, 1H, CHO),
- 7.74 (d, 1H, J = 8.5 Hz, C-6 aryl H),
- 6.47 (dd, 1H, J = 8.5, 2.2 Hz, C-5 aryl H),
- 6.39 (d, 1H, J = 2.1 Hz, C-3 aryl H),
- 3.88 (s, 3H, OCH$_3$),
- 0.99 (s, 9H, SiC(CH$_3$)$_3$),
- 0.26 (s, 6H, Si(CH$_3$)$_2$)

13C NMR (100 MHz, CDCl$_3$), δ:

- 187.7 (C=O),
- 163.4,
- 162.7,
- 129.9,
- 119.2,
- 112.2,
- 103.1,
- 55.2 (OCH$_3$),
- 25.3 (SiC(CH$_3$)$_3$),
- 17.9 (SiC(CH$_3$)$_3$),
- 1167 (s),
- 779 (s)

FTIR (neat), cm$^{-1}$:

- 2955 (m), 2856 (m), 1668 (m, C=O), 1598 (s),
- 1167 (s),
- 779 (s)

HRMS (CI):

Calc'd for C$_{14}$H$_{22}$O$_3$Si [M]$^+$: 266.1338

Found: 266.1331

TLC (30% EtOAc in hexanes), $R_f$: 0.76
n-Butyllithium (555 µL of a 2.35 M solution in hexanes, 1.30 mmol, 1.1 equiv) was added to a degassed, cooled (−78 °C) solution of diisopropylamine (200 µL, 1.42 mmol, 1.2 equiv) in tetrahydrofuran (4.2 mL). The resulting solution was warmed to 0 °C for 10 min and was then cooled to −78 °C. A solution of amine 110 (219 mg, 1.18 mmol, 1 equiv) in THF (0.8 mL) was added to the reaction mixture. After 50 min, aldehyde 172 (377 mg, 1.41 mmol, 1.2 equiv) was added to the reaction mixture. After an additional 45 min, the reaction mixture was poured into water (10 mL), and the product was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (60–70% ethyl acetate–hexanes) afforded the aldol adduct 173 (434 mg, 81%) as an airy white solid.

**1H NMR (300 MHz, CDCl₃), δ:**

- 7.46 (d, 1H, J = 8.3 Hz, C-6 aryl H), 6.47 (dd, 1H, J = 8.2, 2.2 Hz, C-5 aryl H), 6.34 (d, 1H, J = 2.2 Hz, C-3 aryl H), 5.96 (s, 1H, CHO), 3.70 (s, 3H, OCH₃), 2.76 (ABq, 2H, J = 13.5 Hz, Δν = 204.0 Hz, CH₂N(CH₃)₂), 2.26 (s, 6H, N(CH₃)₂), 1.63 (s, 3H, CH₃), 1.57 (s, 3H, CH₃), 0.98 (s, 9H, SiC(CH₃)₃), 0.20 (s, 3H, Si(CH₃)₂)

**13C NMR (100 MHz, CDCl₃), δ:**

- 163.1 (C(O)C=C), 161.3 (C=O), 156.1, 155.6, 126.3, 124.4, 113.2, 110.4, 104.9, 103.2, 63.4,
FTIR (neat), cm\(^{-1}\):

3183 (w, OH), 2954 (m), 2859 (m), 1730 (s, C=O), 1607 (m), 1499 (m), 1392 (m), 1273 (s), 1202 (s), 1032 (m), 842 (s)

HRMS (FAB):

Calc'd for C\(_{23}\)H\(_{38}\)NO\(_6\)Si [M + H]\(^+\): 452.2468

Found: 452.2460

TLC (EtOAc), \(R_f\):

0.34

58.9, 55.0, 44.4 (N(CH\(_3\))\(_2\)), 26.4, 25.5, 23.0, 18.0, -4.6 (Si(CH\(_3\))\(_2\)), -4.6 (Si(CH\(_3\))\(_2\))
Trifluoroacetic acid (7.0 μL, 91.1 μmol, 1.1 equiv) was added to an ice-chilled solution of alcohol 173 (37.4 mg, 82.8 μmol, 1 equiv) in dichloromethane (1.5 mL). After 5 min, Dess–Martin periodinane (56 mg, 132 μmol, 1.6 equiv) was added to the reaction mixture, and the resulting slurry was stirred vigorously at 23 °C. After 20 min, the reaction mixture was diluted with diethyl ether (4 mL) and a 1:1 mixture of saturated aqueous sodium bicarbonate solution and saturated aqueous sodium thiosulfate solution (5 mL) and was stirred at 23 °C. After 30 min, the layers were separated, and the aqueous layer was further extracted with diethyl ether (2 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (ethyl acetate) provided ketone 174 (28.7 mg, 77%) as a yellow oil.

$^1$H NMR (300 MHz, CDCl$_3$), δ:

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$^{13}$C NMR (100 MHz, CDCl$_3$), δ:

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FTIR (neat), cm$^{-1}$:

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HRMS (FAB): 1261 (s), 1206 (s), 839 (s)
Calc'd for C_{23}H_{35}NO_6Si [M]^+: 449.2234
Found: 449.2231

TLC (EtOAc), R_f: 0.21
Tetrabutylammonium fluoride (55 µL of a 1.0 M solution in THF, 55.0 µmol, 1.1 equiv) was added to an ice-chilled solution of alcohol 173 (22.6 mg, 50.0 µmol, 1 equiv) in tetrahydrofuran (2.0 mL). After 15 min, the reaction mixture was diluted with water (5 mL), and the product was extracted with dichloromethane (3 × 5 mL). The combined organics were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (5% methanol–dichloromethane) provided diol 175 (13.8 mg, 82%) as a white solid.

$^1$H NMR (300 MHz, CDCl$_3$), δ: 7.44 (d, 1H, $J = 8.2$ Hz, C-6 aryl H), 6.44 (dd, 1H, $J = 8.2, 2.3$ Hz, C-5 aryl H), 6.38 (d, 1H, $J = 2.3$ Hz, C-3 aryl H), 5.99 (s, 1H, CHOCH), 3.67 (s, 3H, OCH$_3$), 2.80 (ABq, 2H, $J = 13.6$ Hz, $\Delta \nu = 209.3$ Hz, CH$_2$N(CH$_3$)$_2$), 2.27 (s, 6H, N(CH$_3$)$_2$), 1.64 (s, 3H, CH$_3$), 1.58 (s, 3H, CH$_3$)

$^{13}$C NMR (100 MHz, CD$_3$OD), δ: 165.7 (C(O)C=C=), 163.6 (C=O), 159.3, 157.9, 127.7, 123.2, 114.1, 107.1, 106.9, 99.8, 64.3, 58.9, 55.7, 44.9 (N(CH$_3$)$_2$), 26.8 (CH$_3$), 23.4 (CH$_3$)

FTIR (neat), cm$^{-1}$: 3194 (w, OH), 1725 (s, C=O), 1632 (m), 1456 (m), 1275 (m), 1204 (m), 1024 (s), 835 (m)

HRMS (CI): Calc'd for C$_{17}$H$_{24}$NO$_6$ [M + H]$^+$: 338.1603

Found: 338.1598

TLC (EtOAc), $R_f$: 0.14
A buffered solution of hydrogen fluoride•pyridine in tetrahydrofuran was prepared by the addition of 70% hydrogen fluoride•pyridine (500 µL) to a solution of pyridine (2.0 mL) in THF (5.0 mL) at 0 °C. A 130-µL aliquot was added to a solution of ketone 174 (6.3 mg, 14 µmol, 1 equiv) in tetrahydrofuran (1.0 mL) at 23 °C. After 15 min, the reaction mixture was diluted with water (5 mL), and the product was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated to a volume of approximately 500 µL. The solution (pyridine) was then concentrated to dryness from benzene (2 × 2 mL) to afford the crude phenol 176 (1.9 mg, ~40%).

$^1$H NMR (300 MHz, CDCl$_3$), δ: 7.72 (d, 1H, $J = 8.6$ Hz, C-6 aryl H), 6.41 (dd, 1H, $J = 8.6, 2.2$ Hz, C-5 aryl H), 6.30 (d, 1H, $J = 2.2$ Hz, C-3 aryl H), 3.75 (s, 3H, OCH$_3$), 3.37 (s, 2H, CH$_2$N(CH$_3$)$_2$), 2.26 (s, 6H, N(CH$_3$)$_2$), 1.83 (s, 6H, CH$_3$)

FTIR (neat), cm$^{-1}$: 2927 (w), 1723 (s, C=O), 1609 (m), 1571 (m), 1374 (m), 1205 (s), 1112 (m), 836 (m)

TLC (EtOAc), $R_f$: 0.07
Sodium hydride (7.7 mg of a 60% dispersion in mineral oil, 192 µmol, 5.5 equiv) was added to an ice-chilled solution of alcohol 173 (15.8 mg, 35 µmol, 1 equiv) in tetrahydrofuran (1.5 mL). After 55 min, dimethylcarbamyl chloride (39 µL, 420 µmol, 12.0 equiv) was added, and the reaction mixture was stirred at 0 °C for 20 min and was then warmed to 23 °C. After 2.5 h, the reaction mixture was quenched upon the addition of water (5 mL), and the product was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over sodium sulfate and were concentrated. Purification of the residue by flash chromatography (5% methanol–dichloromethane) afforded carbamate 183 (7.1 mg, 39%) as a yellow oil, along with alcohol 173 (4.5 mg, 28%).

\(^1\)H NMR (300 MHz, CDCl\(_3\)), \(\delta\):

- 7.41 (d, 1H, \(J = 8.4\) Hz, C-6 aryl H), 6.90 (s, 1H, CHOR), 6.44 (dd, 1H, \(J = 8.4, 2.2\) Hz, C-5 aryl H), 6.32 (d, 1H, \(J = 2.2\) Hz, C-3 aryl H),
- 3.74 (s, 3H, OCH\(_3\)), 3.44 (ABq, 2H, \(J = 14.8\) Hz, CH\(_2\)N(CH\(_3\))\(_2\)),
- 3.02 (br, 3H, C(O)N(CH\(_3\))\(_2\)), 2.92 (br, 3H, C(O)N(CH\(_3\))\(_2\)),
- 2.20 (s, 6H, CH\(_2\)N(CH\(_3\))\(_2\)), 1.69 (s, 3H, CH\(_3\))
- 1.61 (s, 3H, CH\(_3\)), 0.97 (s, 9H, SiC(CH\(_3\))\(_3\)),
- 0.19 (s, 6H, Si(CH\(_3\))\(_2\))

\(^13\)C NMR (100 MHz, CDCl\(_3\)), \(\delta\):

- 165.8, 159.7, 156.5, 156.2, 155.5, 128.2,
FTIR (neat), cm⁻¹:
2935 (m), 1733 (s, C=O), 1706 (s, C=O), 1388 (s), 1201 (s), 1179 (s), 980 (m), 842 (s)

HRMS (FAB):
Calc’d for C₂₆H₄₃N₂O₇Si [M + H]⁺: 523.2839
Found: 523.2823

TLC (EtOAc), Rᵣ:
0.14
References and Notes


13. As an exception, see reference 10.


16. The yield of this sequence (13→14) has been improved to 72%, see: Myers, A. G.; Zheng, B. J. Am. Chem. Soc. 1996, 118, 4492–4493.


54. In the experimental section of reference 53b, this reagent is "assumed to be Pb₂OH(OAc)₃, Baker & Adamson reagent." Although lead(II) acetate hydroxide [Pb₂OH(OAc)₃] is identified as "basic lead acetate" in the Dictionary of Inorganic Compounds, this reagent is apparently not commercially available. Lead subacetate [Pb(OAc)₂•2Pb(OH)₃], which is commercially available, is also referred to as "basic lead acetate." The exact composition of the reagent they utilized is unclear.


64. Muxfeldt, H.; Kreutzer, A. Naturwissenschaften 1959, 46, 204–205.


84. Both (R)- and (S)-Mosher ester derivatives were prepared for comparative analysis.


Appendix

Catalog of Spectra
11

TBSO
\[ \text{Br} \]
\[ \text{H} \]
\[ \text{TMS} \]

12

TBSO
\[ \text{C} \]
\[ \text{OH} \]
\[ \text{TMS} \]
15
160

161