

CYCLIC ENAMINONES: SYNTHONS FOR PIPERIDINE CONTAINING NATURAL  
PRODUCTS AND NATURAL PRODUCT ANALOGS

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## Abstract

### Cyclic Enaminones: Synthons for Piperidine Containing Natural Products and Natural Product Analogs

Cyclic enaminones are important synthetic intermediates for the preparation of piperidine containing natural products and target molecules such as drugs, which require a piperidine moiety for bioactivity. They are valuable synthons because of their unique reactivity and chemical stability. In this thesis, I developed novel chemistry from which to derivatize enaminones, and additionally I utilized cyclic enaminones as synthons for the preparation of natural product derivatives.

I discovered that  $\alpha,\beta$ -unsaturated aldehydes add to the  $\alpha$ -carbon of enaminones in the presence of organocatalysts and thereby, introduce aliphatic  $\alpha$ -branched substituents; a reaction that was previously very difficult to accomplish. The chiral reaction products were obtained in good- to excellent yields and with high enantiopurity. The absolute stereochemistry of the reaction products was also determined.

I prepared analogues of the cytotoxic phenanthropiperidines tylophorine and boehmeriasin A, and I synthesized dihydrolyfoline, a member of a group of natural biphenylquinolizidine lactone alkaloids that possess a wide variety of bioactivities. The natural occurring cytotoxic phenanthropiperidines suffer from poor physicochemical properties and adverse side effects. Thus, the target molecules were designed to mitigate the unwanted side effects by improving their physicochemical properties. I devised short, concise routes toward the synthesis of a library of simplified tylophorine analogs, as well

as 15-hydroxyboehmeriasin A. The tylophorine analog library was screened for antiproliferative activity against several cancer cell lines and thus, insight was gained into the structure-activity relationships of this class of compounds including the indication that an intact indolizidine moiety is critical for high potency of tylophorine analogues. 15-Hydroxyboehmeriasin A was prepared and evaluated for cytotoxicity against the A549 human lung carcinoma cell line. An observed  $GI_{50}$  of 81 nM demonstrates that the addition of the 15-hydroxyl group was not detrimental to cytotoxicity and that this position should be explored for further modifications in efforts to improve the physicochemical properties. In addition I prepared ( $\pm$ )-dihydrolyfoline in a concise manner from a bicyclic enaminone precursor using a biomimetic oxidative biaryl coupling approach as the key reaction step.

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| 6,7,10,11-Tetramethoxy-2-methyl-1,2,3,4-tetrahydrodibenzo-<br>[ <i>f,h</i> ]isoquinoline ( <b>2.116</b> )  | 129 |

|   |     |
|---|-----|
| 6,7,10,11-Tetramethoxy-2-phenethyl-1,2,3,4-tetrahydrobenzo-<br>[f,h]isoquinoline ( <b>2.117</b> )   | 129 |
| <i>tert</i> -Butyl 2-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[f,h]-<br>isoquinolin-2(1 <i>H</i> )-yl)ethyl)carbamate ( <b>2.118</b> )          | 129 |
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| 3-Phenyl-2-tosyl-1,2-oxaziridine ( <b>2.129</b> )   | 136 |
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| ( <i>R</i> )-2-(1-( <i>tert</i> -Butoxycarbonyl)piperidin-2-yl)acetic acid ( <b>2.138</b> )   | 139 |
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### Chapter 3

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### List of abbreviations

|           |                                      |
|-----------|--------------------------------------|
| Ac        | acetyl                               |
| AIBN      | 2,2'-azobisisobutyronitrile          |
| <i>aq</i> | aqueous                              |
| Ar        | aryl                                 |
| BBB       | blood brain barrier                  |
| Bn        | benzyl                               |
| Boc       | <i>tert</i> -butoxycarbonyl          |
| BOM       | benzyloxymethyl                      |
| br        | broad                                |
| BRSM      | based on recovered starting material |
| BTEAC     | benzyltriethylammonium chloride      |
| bu        | butyl                                |
| CDK       | cyclin dependant kinase              |
| CNS       | central nervous system               |
| COX       | cyclooxygenase                       |
| d         | doublet                              |
| dba       | dibenzylideneacetone                 |
| DCC       | dicyclohexylcarbodiimide             |
| DCE       | 1,2-dichloroethane                   |
| DIBAL-H   | diisobutylaluminum hydride           |
| DMAP      | 4-dimethylaminopyridine              |

|       |   |
|-------|---|
| DMF   | dimethylformamide                             |
| DME   | dimethoxyethane                               |
| DMS   | dimethylsulfide                               |
| DMSO  | dimethylsulfoxide                             |
| DNA   | deoxyribonucleic acid                         |
| dppf  | 1,1'-bis(diphenylphosphino)ferrocene          |
| d.r.  | diastereomeric ratio                          |
| EDCI  | 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide |
| e.r.  | enantiomeric ratio                            |
| Et    | ethyl   |
| EtOAc | ethyl acetate                                 |
| EtOH  | ethanol                                       |
| GI    | growth inhibition                             |
| h     | hour  |
| HBD   | hydrogen bond donor                           |
| Hex   | hexanes                                       |
| HIF   | hypoxia inducible factor                      |
| HMDS  | hexamethyldisilazide                          |
| HMQC  | heteronuclear multiple-quantum correlation    |
| HPLC  | high pressure liquid chromatography           |
| IC    | inhibitory concentration                      |
| IG    | immunoglobulin                                |
| IL    | interleukin                                   |
| IR    | infrared                                      |

|                  |  |
|------------------|--|
| <i>i</i> -Pr     | isopropyl                                    |
| K <sub>D</sub>   | dissociation constant                        |
| LDA              | lithium diisopropylamide                     |
| LPS              | lipopolysaccharide                           |
| LUMO             | lowest unoccupied molecular orbital          |
| m                | multiplet                                    |
| MAO              | monoamine oxidase                            |
| <i>m</i> CPBA    | <i>meta</i> -chloroperoxybenzoic acid        |
| Me               | methyl                                       |
| MeOH             | methanol                                     |
| MDR              | multidrug resistant                          |
| μM               | micromolar                                   |
| min              | minute                                       |
| MPP <sup>+</sup> | 1-methyl-4-phenylpyridinium                  |
| MPTP             | 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine |
| Ms               | mesyl  |
| MTPA             | methoxytrifluoromethylphenylacetic acid      |
| MW               | molecular weight                             |
| NBS              | <i>N</i> -bromosuccinimide                   |
| NCI              | National Cancer Institute                    |
| NCS              | <i>N</i> -chlorosuccinimide                  |
| NF               | nuclear factor                               |
| NHC              | <i>N</i> -heterocyclic carbene               |
| NIMH             | National Institute of Mental Health          |



|                |   |
|----------------|---|
| nM             | nanomolar   |
| NMM            | <i>N</i> -methylmorpholine                                  |
| NMR            | nuclear magnetic resonance                                  |
| PDSP           | psychoactive drug screening program                         |
| P-gp           | P-glycoprotein  |
| Ph             | phenyl  |
| PIFA           | (bis(trifluoroacetoxy)iodo)benzene                          |
| PK             | pharmacokinetic   |
| PPA            | polyphosphoric acid   |
| PPTS           | pyridinium <i>para</i> -toluenesulfonate                    |
| PSA            | polar surface area  |
| <i>p</i> -TSA  | <i>para</i> -toluenesulfonic acid                           |
| RNA            | ribonucleic acid  |
| rt             | room temperature  |
| s              | singlet   |
| SAR            | structure-activity relationship                             |
| sat            | saturated   |
| SOMO           | singly occupied molecular orbital                           |
| <i>S</i> -phos | dicyclohexyl(2',6'-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine |
| t              | triplet   |
| <i>t</i> -Bu   | <i>tert</i> -butyl  |
| TBAF           | tetra- <i>N</i> -butylammonium fluoride                     |
| TBDPS          | <i>tert</i> -butyldiphenylsilyl                             |
| TBS            | <i>tert</i> -butyldimethylsilyl                             |

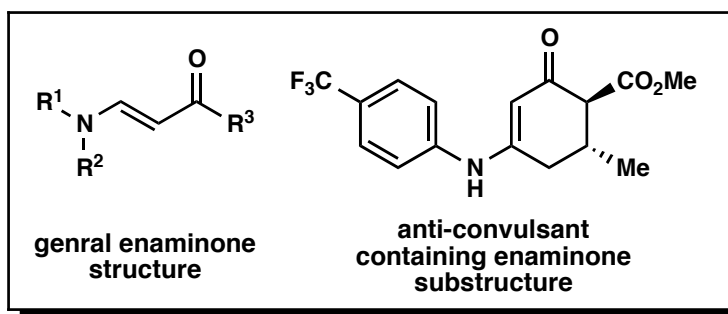
|       |  |
|-------|--|
| TEA   | triethylamine                                |
| TES   | triethylsilyl                                |
| Tf    | trifluoromethanesulfonyl                     |
| TFA   | trifluoroacetic acid                         |
| TFAA  | trifluoroacetic anhydride                    |
| THF   | tetrahydrofuran                              |
| TMEDA | <i>N,N,N',N'</i> -tetramethylethylenediamine |
| TMS   | trimethylsilyl                               |
| TMV   | tobacco mosaic virus                         |
| TNF   | tumor necrosis factor                        |
| VEGF  | vascular endothelial growth factor           |

## Chapter 1

### Enantioselective Michael Addition of Enaminones Using Organocatalysts

#### 1.1 Introduction to Cyclic Enaminones

Enaminones (Figure 1-1) can be described as  $\beta$ -acyl enamines or an amide with an interpolated alkene (vinylogous amide). The reactivity profile of these vinylogous amides is quite different than that of a traditional enamine, which readily decomposes under oxidative or hydrolytic conditions.<sup>1</sup> On the other hand, enaminones are much more robust, stable, and easily isolated. While not quite as robust as a traditional amide, the conjugation of the enamine to the carbonyl reduces the reactivity giving it a unique reactivity profile. In addition to the distinct reactivity, enaminones have shown activity as P-gp modulators and anti-convulsants.<sup>2-5</sup>

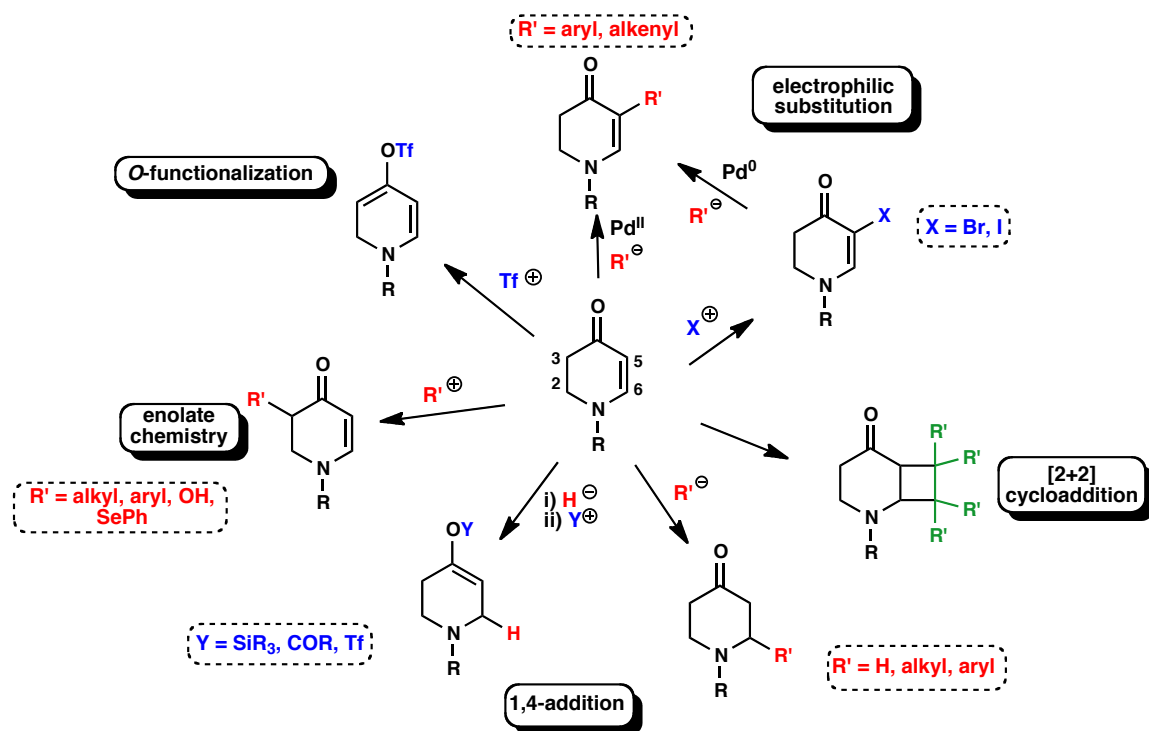


**Figure 1-1.** General structure of the enaminone and structure of an enaminone with anti-convulsant activity.

Cyclic enaminones, especially six-membered enaminones, are extremely versatile intermediates for the synthesis of natural products and medicinal agents.<sup>2,6-8</sup> Lastly, the

piperidine functional group is present in classes of bioactive natural products, such as the indolizidines and quinolizidines.<sup>9,10</sup>

The synthetic utility of the enaminone becomes clear when considering each component separately (amine, enamine, enone, and alkene). There are four nucleophilic sites and two electrophilic sites that serve as functional handles for modification. Studies regarding the reactivity of the enaminone have provided a plethora of chemoselective reactions (*i.e.* *N*-functionalization,<sup>11,12</sup> *O*-functionalization,<sup>13-15</sup> C3,<sup>16-19</sup> C4 [1,2-addition],<sup>20,21</sup> C5,<sup>22-24</sup> and C6 [conjugate addition]<sup>25-30</sup> etc., as depicted in Figure 1-2.



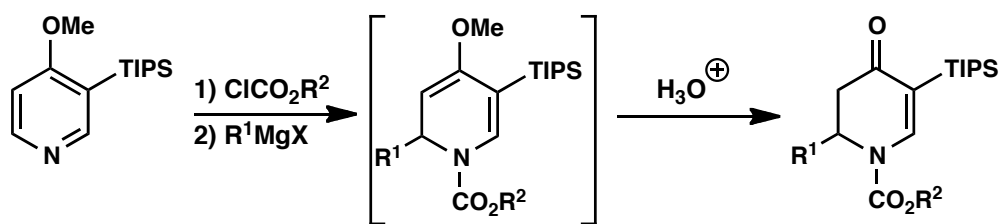
**Figure 1-2.** Chemoselective transformations of the six-membered cyclic enaminone.

Many approaches have been developed to synthesize the 6-membered enaminone (Scheme 1-1). Comins and coworkers have set the bar for the construction of

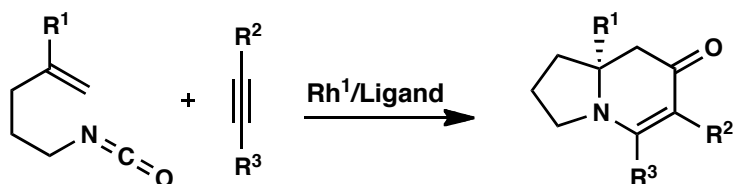
asymmetrical enamines employing *N*-acylpyridinium species using a chiral auxiliary approach.<sup>31</sup> This method has stood the test of time and been used in several natural product syntheses.<sup>6,8,32,33</sup> More recently, [2+2+2] cycloadditions have been developed by the Rovis group. In this case, alkynes undergo the cycloaddition with alkenylisocyanates in the presence of chiral rhodium catalysts.<sup>34-38</sup> While this approach is not limited to indolizidine synthesis, there is only one report of a quinolizidine synthesis.<sup>38</sup> Another method that predates the previous two is the hetero-Diels-Alder reaction of imines with Danishefsky's diene.<sup>39-45</sup> Recently Georg and coworkers developed a chiral pool approach using  $\beta$ -amino acids to construct the 6-membered enamines (both indolizidines and quinolizidines).<sup>46,47</sup>

**Scheme 1-1.** General approaches for the synthesis of 6-membered enamines

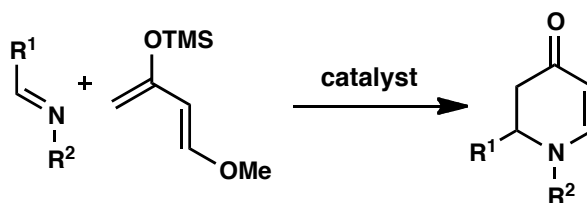
**Comins**



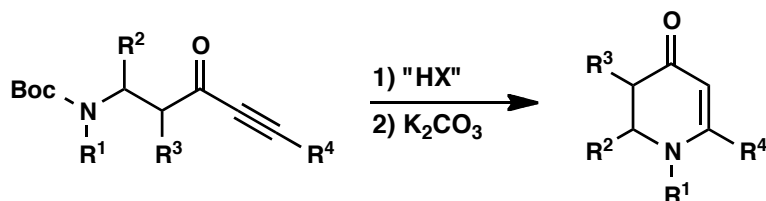
**Rovis**



### Hetero Diels-Alder



### Georg

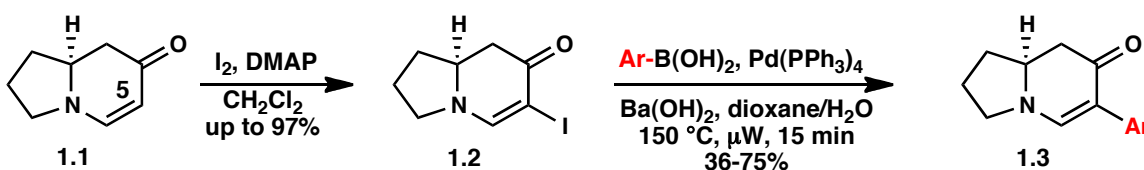


In order to convert the enaminone scaffold to a precursor of the phenanthrene system of the phenanthropiperidine alkaloids a C5-arylated enaminone (see Scheme 1-2) is required. In pursuit of this goal, two methods were developed for the C5-arylation of enaminones.<sup>22,23</sup> Both of the methods relied on palladium and organoboron chemistry (Suzuki-Miyaura reaction) for the C-C bond formation. Boronic acids and esters are the most commonly used coupling partner and have some inherent stability advantages as compared to their organometallic counterparts such as Mg or Zn. Furthermore, they are sufficiently reactive under mild conditions, non-toxic, and thousands are commercially available.

The first method relied on the reactivity of the enaminone to pre-activate the enaminone (Scheme 1-2) for a subsequent metal-catalyzed cross-coupling reaction. The nitrogen's lone pair of electrons is conjugated with the  $\pi$ -system, which explains the

positions enhanced nucleophilicity of the C-5 position. Using enaminone **1.1** as a model substrate, halogenation at the C5 position was explored. It was discovered that treatment of the enaminone with iodine and DMAP furnished the  $\alpha$ -iodoenaminone **1.2** in near quantitative yields. Bromination is also possible, but given that C–I bonds are more susceptible to oxidative insertion than C–Br bonds, iodoenaminones were used for the cross coupling. Coupling conditions were found to be optimal using Pd(PPh<sub>3</sub>)<sub>4</sub> at 100 °C

**Scheme 1-2.** First generation  $\alpha$ -arylation of enaminones



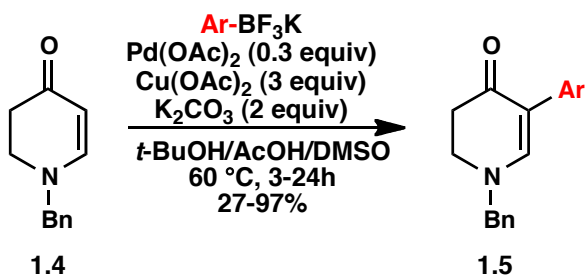
for 14 h. The long reaction times could be avoided using microwave irradiation and a higher temperature (Scheme 1-2), however, a significant amount of degradation was observed with both conditions. Oxidative insertion is the rate-determining step in this reaction, which proceeds best when the electrophile is electron deficient. This is a “Catch-22” situation because the iodine is located at the most electron rich position of the enaminone, which explains why the pre-activation proceeds so smoothly and palladium insertion is less favored. Increasing the reaction temperature and altering the catalyst to have a higher reduction potential can promote oxidative insertion. To this end, the use of the electron-rich ligand *S*-Phos, which accelerates the rate of oxidative addition and promotes reductive elimination, accomplished the goals of decreased reaction times and

proceeding at lower temperatures, but failed to sufficiently increase the yields. Not completely satisfied with those results, a re-examination of the enaminones inherent nucleophilicity led to a more direct approach.

From a mechanistic standpoint, the direct C–H functionalization seemed plausible because once the C–Pd bond is formed from the C–H bond the reaction mechanism is analogous to that of the classical Pd-catalyzed cross coupling. Other than the point at which the C–H donor is introduced, the key distinction between the catalytic cycles is that one begins with a Pd(0) catalyst and the other begins with a Pd(II) catalyst. This distinction is significant, because in both catalytic cycles Pd(0) is generated after the reductive elimination step. In the Pd(II) catalytic cycle, Pd(0) elimination would result in a dead end and the catalytic cycle would not continue. Fortunately, this can be remedied by the addition of an external oxidant, which oxidizes Pd(0) to Pd(II), the active catalyst in the catalytic cycle.

The success of this approach ultimately depends on the nucleophilicity of the enaminone, which in theory would give the same palladium species in one step rather than the two-step process. To aid in the electrophilic palladation, an acidic cosolvent was

**Scheme 1-3.** Second-generation  $\alpha$ -arylation of enaminones





added,<sup>48</sup> thus, severely limiting the organometallic coupling partners (such as Grignards or zincates). Molander *et al.* found that organotrifluoroborates are robust equivalents of organoboronic acids, and thus were chosen as suitable coupling partners.<sup>49</sup> After extensive experimentation, it was discovered that Pd(OAc)<sub>2</sub>, Cu(OAc)<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, at 60 °C, with the *t*-BuOH/AcOH/DMSO solvent system (Scheme 1-3) gave the  $\alpha$ -arylated enaminone **1.5** (Scheme 1-3). This second generation protocol generally has an excellent scope for aromatic trifluoroborates (i.e., electron donating, electron withdrawing, phenols, aryl halides, etc.), however, this method does have some drawbacks. Heteroaromatic, alkynyl, alkenyl, and alkyl trifluoroborates did not couple to the enaminone. With methodology studies underway to couple alkenyl<sup>24</sup> groups to the enaminone, a systematic investigation to add alkyl groups to the enaminone was undertaken. The previous unsuccessful attempts with transition metal catalysis led us to pursue organocatalysis as a viable option to not only add alkyl functionalities to the enaminone, but also to explore enantioselective additions.

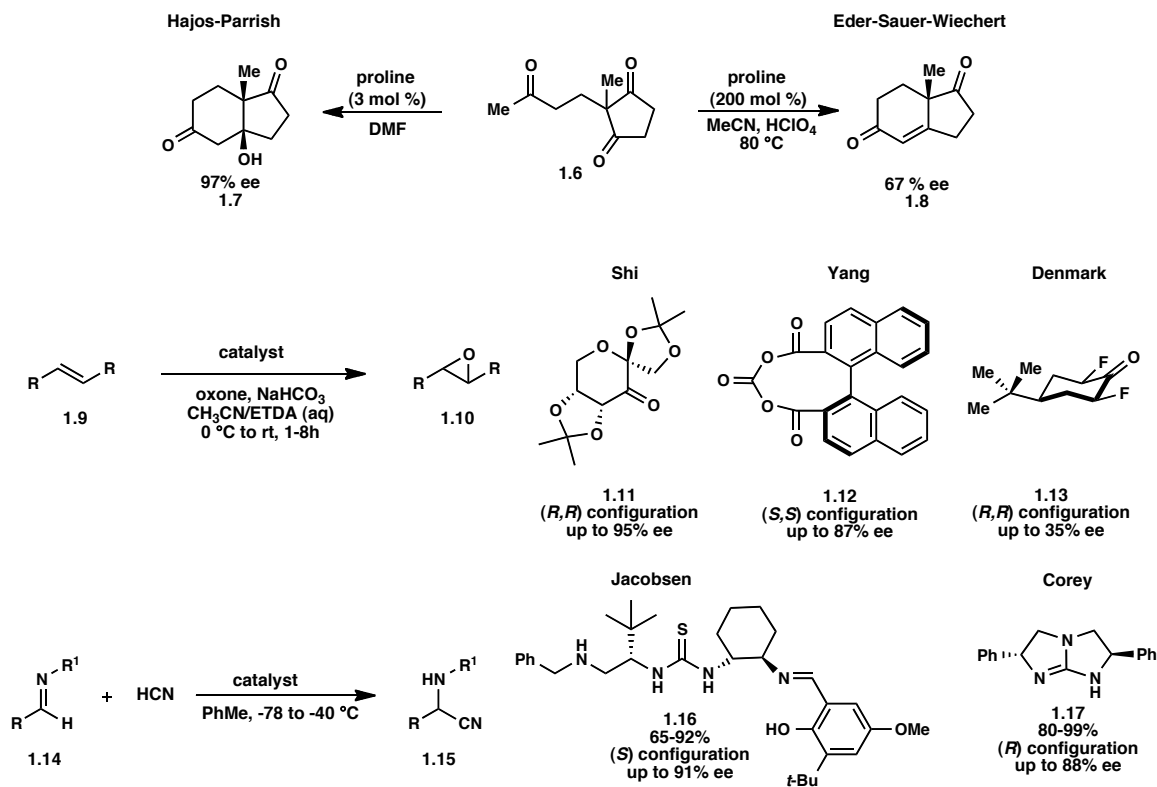
## 1.2 Historical background of organocatalysis

Chemical transformations that use organic catalysts, or organocatalysts, have been periodically documented over the last half-century.<sup>50-56</sup> However, it was not until the late 1990's that the field of organocatalysis was 'born.'<sup>57</sup> It is now widely accepted that organocatalysis has emerged into its own branch of enantioselective catalysis/synthesis.<sup>57</sup>

Between 1968 and 1997, there were only a few reports of the use of small organic molecules as catalysts (Hajos-Parrish reaction being the most famous), but these reactions

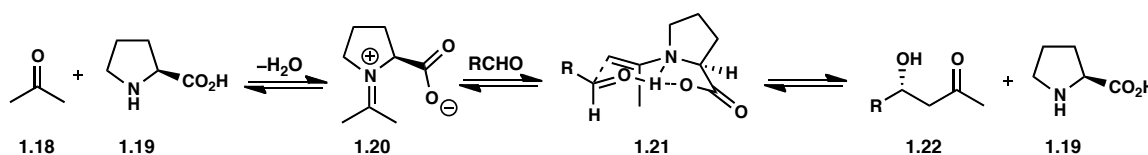
were viewed as unique chemical reactions rather than integral parts of a larger field.<sup>50-56</sup> It wasn't until the late 1990's that the field began to evolve when Shi,<sup>58</sup> Denmark,<sup>59</sup> and Yang<sup>60</sup> independently demonstrated that the epoxidation of alkenes could be catalyzed by enantiomerically pure chiral ketones. Following those reports Jacobsen<sup>61,62</sup> and Corey<sup>63</sup> reported hydrogen-bonding catalysis in asymmetric Strecker reactions. Miller then achieved the catalytic enantioselective kinetic resolution of alcohols using peptides.<sup>64</sup> These results demonstrated that small organocatalysts could be used to solve important problems in organic synthesis.

**Scheme 1-4.** Early reports of organocatalytic reactions



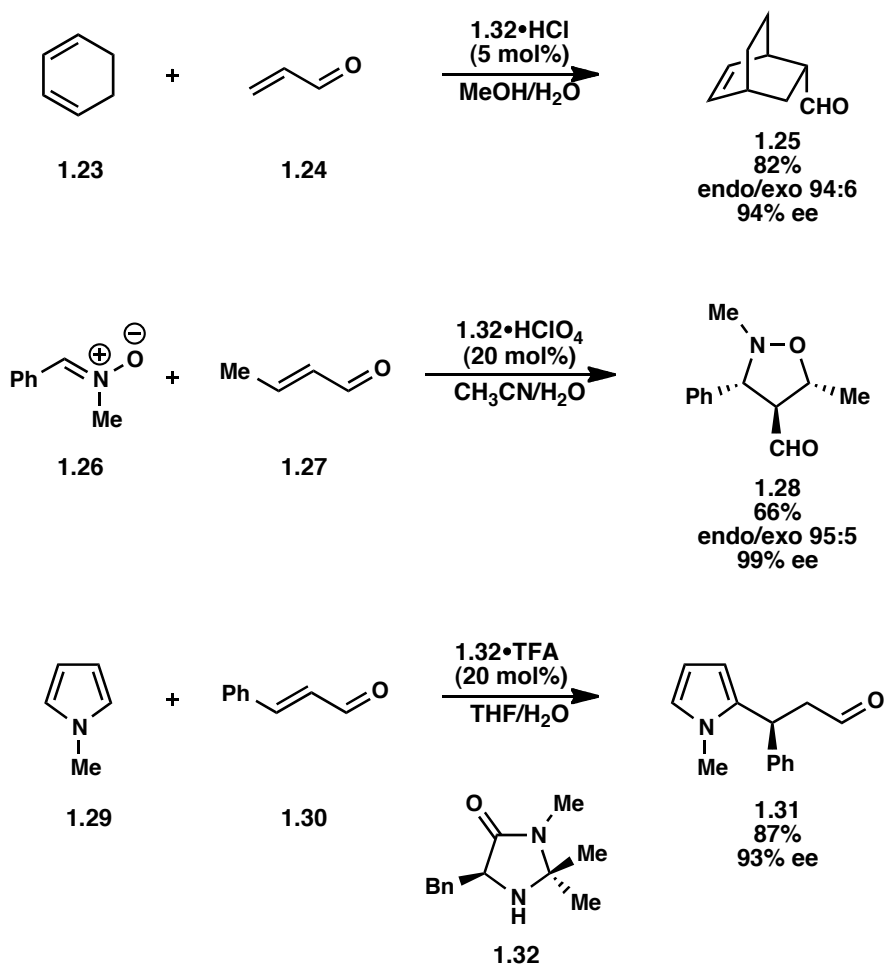
In 2000, two reports published simultaneously by List *et al.*<sup>65</sup> and MacMillan *et al.*<sup>66</sup> changed the landscape of organocatalysis. The work of List and coworkers showed that the Hajos-Parrish reaction could be applied to transformations that are more pertinent, such as the aldol reaction (Scheme 1-5). Another major point of this work is that small organic molecules could catalyze chemical reactions in a similar manner as much larger biomolecules.

**Scheme 1-5.** Proline catalyzed asymmetric aldol reaction



MacMillan introduced the imidazolidinone catalysts, which exploit the reversible condensation of a chiral amine with an aldehyde to form an iminium ion intermediate (Scheme 1-6). In this system, a rapid equilibrium exists between an electron-deficient and an electron-rich state, which effectively lowers the LUMO energy of the  $\pi$ -system and enhances its susceptibility toward nucleophilic attack.<sup>67,68</sup> Importantly, further studies by MacMillan and coworkers established the effectiveness of the readily available chiral imidazolidinones such as **1.32** (Scheme 1-6) to promote mechanistically distinct transformations of  $\alpha,\beta$ -unsaturated aldehydes in a highly enantioselective fashion.<sup>69,70</sup> In the ten years since these reports, organocatalysis has exploded into its own field of research.

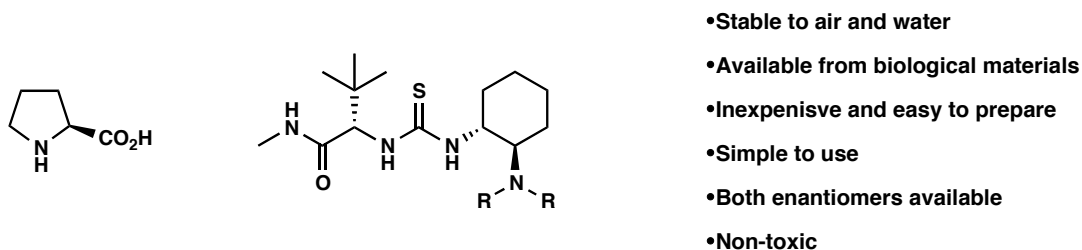
### Scheme 1-6. Imidazolidinone catalyzed transformations



### 1.3 Advantages of organocatalysis and organocatalysts

There are fundamental advantages of organocatalysis, specifically the ease with which reactions can be carried out (generally not water sensitive) and the availability of the catalysts. Historically, asymmetric catalysis was accomplished with metal-based chiral catalysts. Through these catalysts a myriad of asymmetric reactions have been

developed. In fact the 2010 Nobel prize in chemistry was awarded to Knowles, Noyori, and Sharpless for their work on metal-based chirally catalyzed reactions.<sup>71</sup> The major drawback to organometallic systems is that they can be quite sensitive to oxygen and water in solvents and the atmosphere. Organocatalysts differ from metal-based catalysts



**Figure 1-3.** Typical advantages of using organocatalysts.

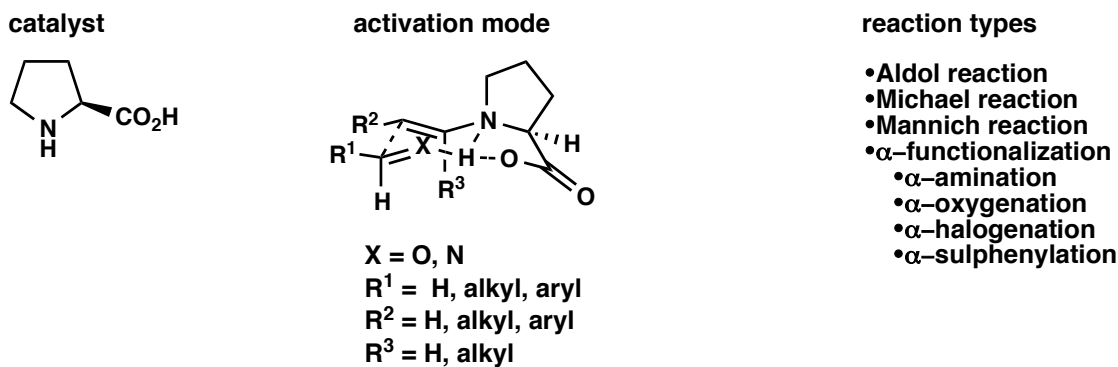
in that they bring time and energy efficiency,<sup>57,72</sup> and mild reaction conditions to the lab.<sup>73</sup> Organocatalysts typically don't require special reaction conditions/techniques because they are generally insensitive to oxygen and moisture. Organocatalysts are typically prepared from organic molecules that are readily available from biological sources (amino acids, carbohydrates, and hydroxy acids).<sup>73</sup> Therefore organocatalysts are generally inexpensive to prepare and available in a wide range of quantities from small-scale to industrial scale.<sup>74</sup> These small organic molecules are non-toxic and environmentally benign, increasing the safety across all research settings.<sup>75</sup> Given the combination of these factors, it is no surprise that the field has exploded to where it is today.

## 1.4 Generic modes of activation

Central to the success of organocatalysis over the past decade has been the identification of generic modes of activation. A generic mode of activation describes a reactive intermediate that participates in many reactions with high enantioselectivity.<sup>57</sup> Such reactive intermediates arise from the interaction of a single chiral catalyst with a basic functional group such as a ketone, aldehyde, alkene or imine in an organized and predictable manner. The value of these activation modes is that they can now be utilized in the design and development of new enantioselective transformations. Most of the ~150 unique organocatalytic reactions that have been reported since 1998 are founded directly on six activation modes.<sup>72,73,75</sup> The activation modes will be discussed in the following sections.

### 1.4.1 Enamine catalysis

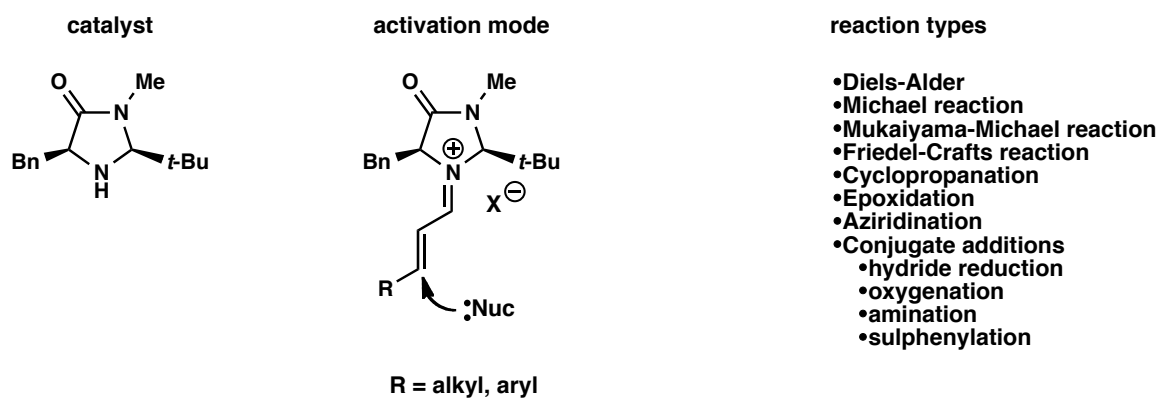
In 1971 two patents were filed on the enantioselective intramolecular aldol reaction catalyzed by proline.<sup>55,56</sup> In 2000, List and coworkers used proline to catalyze the  $\alpha$ -functionalization of carbonyl-containing compounds through enamine catalysis.<sup>65</sup> Mechanistically, the enamine is formed by a reaction between the chiral amine and the carbonyl while simultaneously forming a H-bond with the electrophilic reaction partner. This general mode of activation has now been used in a wide variety of  $\alpha$ -functionalization reactions.<sup>76</sup>



**Figure 1-4.** Enamine catalysis.

### 1.4.2 Iminium catalysis

Iminium catalysis was designed, as opposed to being discovered. This type of activation is based on the principle that chiral amines can function as catalysts for enantioselective transformations that traditionally use Lewis-acids. Mechanistically, the concept is based on the reversible formation of iminium ions from  $\alpha,\beta$ -unsaturated aldehydes and chiral amines, which imitate the  $\pi$ -orbital dynamics that is inherent to Lewis-acid catalysis (LUMO-lowering activation). The traditional catalysts used for this type of transformation are the imidazolidinones, which have now been used in many enantioselective reactions.<sup>77,78</sup>

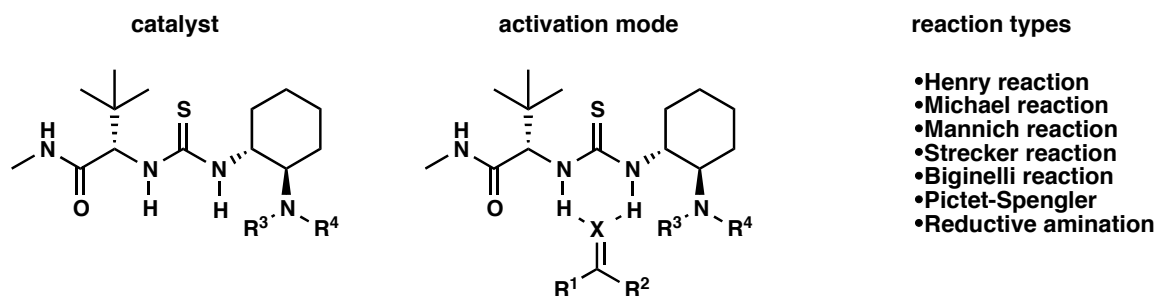


**Figure 1-5.** Iminium ion catalysis.

### 1.4.3 Hydrogen bonding catalysis

In the early 1980's, several researchers discovered that enantioselective catalytic processes could be carried out by activation of a substrate through well-defined H-bonds.<sup>79-81</sup> These well-defined H-bonds formed a highly organized transition state that allowed for the enantioselective transformation.<sup>57</sup> This was corroborated by two reports published independently by Jacobsen<sup>61,62</sup> and Corey<sup>63</sup> on asymmetric variations of the Strecker reaction. These reports were based on the well-defined H-bond interactions between the organocatalysts and the imine electrophile; a previously unaccepted principle that H-bonds were insufficient for activating or directing for use in asymmetric catalysis. In the years since these reports surfaced, the thiourea catalysts have been used for other synthetic reactions.<sup>82</sup> This principle has now been used for more than 30 different asymmetric transformations.<sup>83</sup>

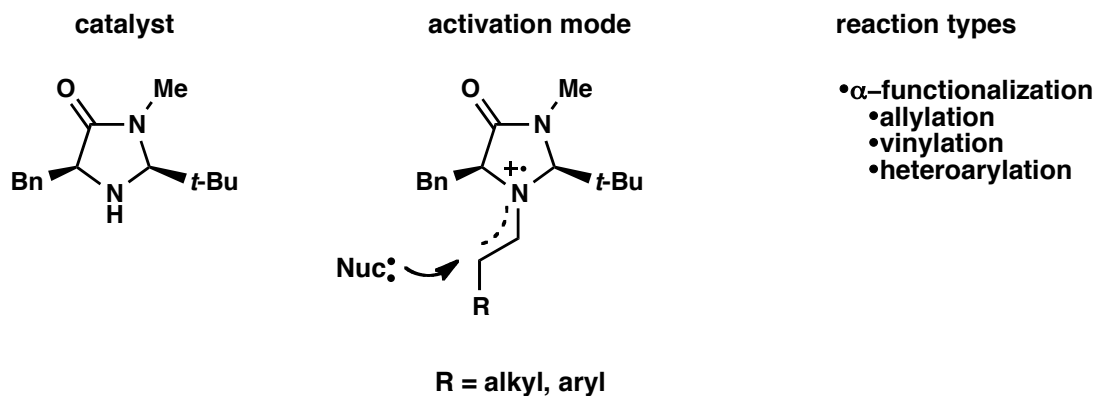




**Figure 1-6.** Hydrogen-bonding catalysis.

#### 1.4.4 SOMO catalysis

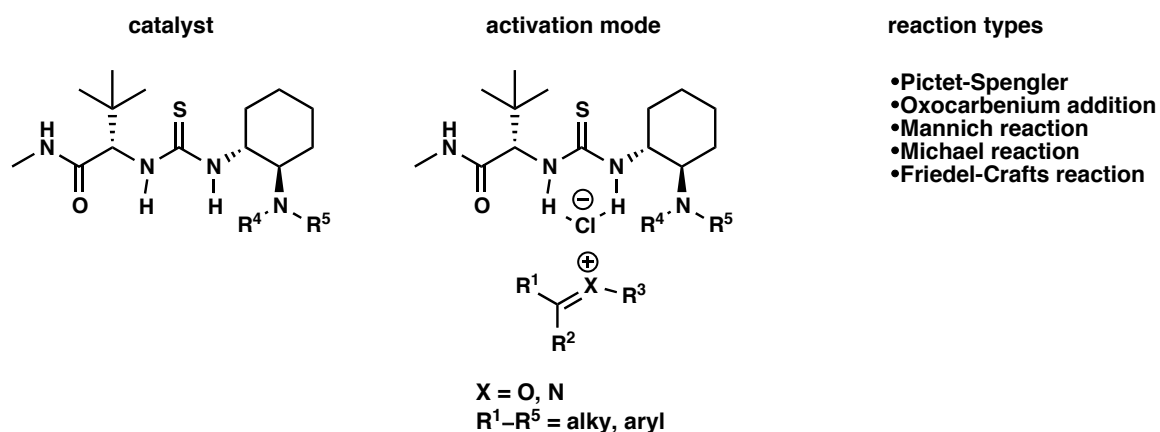
SOMO (singly occupied molecular orbital) catalysis, introduced in 2007 by MacMillan and coworkers, is based on the oxidation of an electron-rich enamine.<sup>57</sup> The single electron oxidation generates a radical cation with three  $\pi$ -electrons (Figure 1-7) which now contains an electrophilic SOMO that can now react with weak carbon-based nucleophiles at the  $\alpha$ -carbon of the enamine, which is a formal alkylation.<sup>84</sup> In a catalytic system, a chiral secondary amine and one-electron oxidant have been extremely successful in the  $\alpha$ -functionalization of carbonyl compounds. This new avenue for catalysis has already supplied a number of enantioselective transformations.<sup>85-87</sup>



**Figure 1-7.** SOMO catalysis.

### 1.4.5 Counterion catalysis

Another form of activation introduced in 2007 is counterion catalysis (Figure 1-8). This method introduced by Jacobsen directs highly enantioselective additions into temporarily generated oxocarbenium and *N*-acyl-iminium ions.<sup>88,89</sup> In this catalytic system, an ion pair is formed when the thiourea catalysts complex together with halide ions using electrostatic interactions forming a chiral complex. Thus, the approach of the nucleophile is biased to a single face by the chiral counterion. Although this type of activation is relatively new, the potential is significant given that the stereochemical information of the catalyst is transferred to the substrate through space interactions, as opposed to through bond interactions.<sup>57</sup>



**Figure 1-8.** Counterion catalysis.

#### 1.4.6 *N*-Heterocyclic carbene catalysis (NHC)

The inversion of chemical reactivity, Umpolung, opens up a new avenue of catalysis. Besides their role as excellent ligands in metal-based catalytic reactions, organocatalytic carbene catalysis has emerged as an exceptionally fruitful research area in synthetic organic chemistry (Figure 1-9).<sup>90-92</sup> In this catalytic system, the nucleophilic carbene attacks an electrophile, such as an aldehyde, and reverses the chemical reactivity turning the aldehyde into the nucleophile. The chiral carbene complex transfers its chirality into the product by biasing the approach of the electrophile for inter- or intramolecular attack. The variety of reactions catalyzed by NHC's has grown immensely over the last five years and the possibilities of combining these with traditional modes of activation will make organocatalytic carbene catalysis, a particular area of interest in the future.

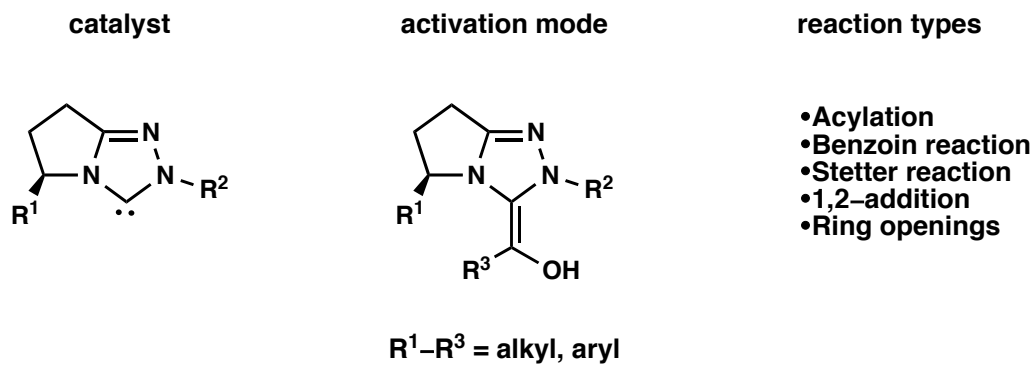


Figure 1-9. NHC catalysis.

## 1.5 Enantioselective Michael additions of enaminones using organocatalysts

### 1.5.1 Strategy and considerations for methodology

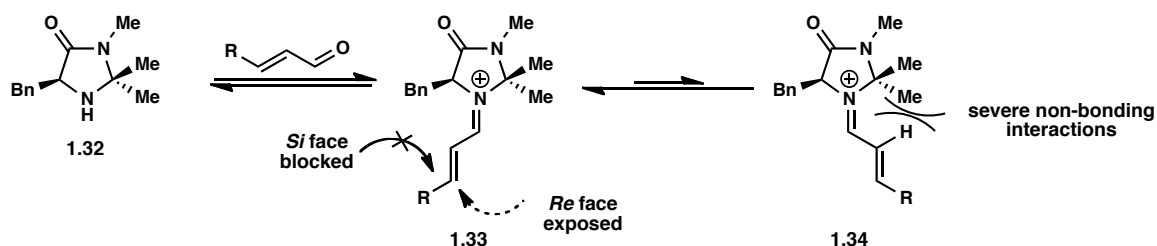
As previously mentioned, functionalization of the C5 position on the enaminone has taken tremendous steps forward in recent years. Two generations of arylation at the C5 position, one using pre-functionalization<sup>23</sup> and the other using direct C-H functionalization,<sup>22</sup> as well as the addition of alkenyl groups<sup>24</sup> have been developed. While the addition of  $sp^2$ -hybridized carbons was extremely successful, the addition of alkyl ( $sp^3$ -hybridized groups) was not successful when using any of the aforementioned methodologies. The success of the direct C-H functionalization hinged upon the nucleophilic character of the enaminone at the C5 position. Keeping that in mind, we sought to develop a suitable chiral electrophile that allows the addition of the enaminone under “mild” conditions. Literature precedent showed that chiral iminium ion

electrophiles could be formed from  $\alpha,\beta$ -unsaturated aldehydes and a chiral imidazolidinone catalyst, and thus was chosen for further evaluation.

### 1.5.2 Development of imidazolidinone catalysts

Imidazolidinone catalysts rely on their ability to effectively and reversibly form a reactive iminium ion with high levels of both configurational control and  $\pi$ -facial discrimination (Scheme 1-7). The activated iminium intermediate **1.33** exists predominantly in the *E*-configuration, to avoid severe non-bonding interactions between the olefinic hydrogen of the substrate and the *geminal* dimethyl groups of the catalyst. Enantioselective bond formation is observed because  $\pi$ -facial discrimination arises from the benzyl blocking the *Si* face, leaving the *Re* face exposed for nucleophilic attack.

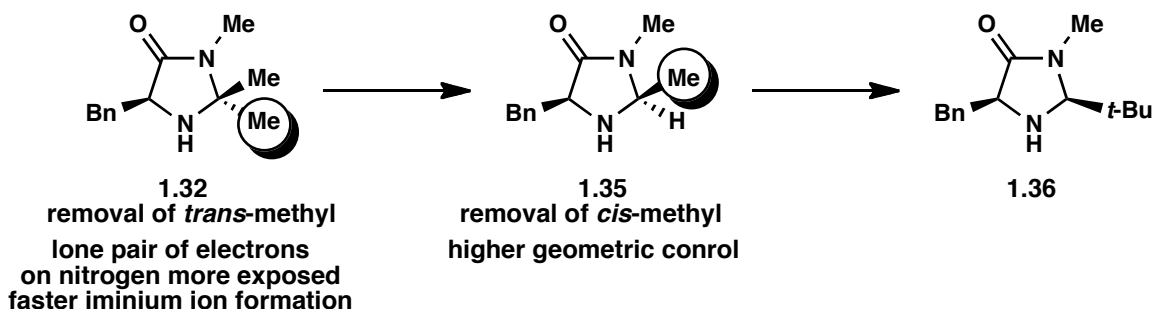
**Scheme 1-7.** Configurational control and  $\pi$ -facial discrimination of imidazolidinones



While catalyst **1.32** was an efficient catalyst for the asymmetric addition of pyrroles to unsaturated aldehydes,<sup>70</sup> the catalytic scope was extended to weaker  $\pi$ -nucleophiles such as furans and indoles. In these situations, catalyst **1.32** gave poor

results in terms of enantioselectivity and reactivity leading to the development of a more versatile and reactive amine catalyst **1.36** (Scheme 1-8). Kinetic studies showed that the reaction rate using catalyst **1.32** was influenced by both the C–C bond forming step and the iminium ion formation.<sup>93</sup> It was then hypothesized that the reaction rate could be greatly enhanced by replacement of the *trans*-methyl (trans to the benzyl) with hydrogen. This reduces the steric congestion around the nitrogen's lone pair resulting in faster iminium ion formation. Replacement of the *cis*-methyl group with a larger substituent, such as a *tert*-butyl, would provide superior geometric control of the iminium ion as well as increased  $\pi$ -facial shielding.<sup>94</sup> This resulted in imidazolidinone catalyst **1.36**.

**Scheme 1-8.** Design of second-generation imidazolidinone catalyst

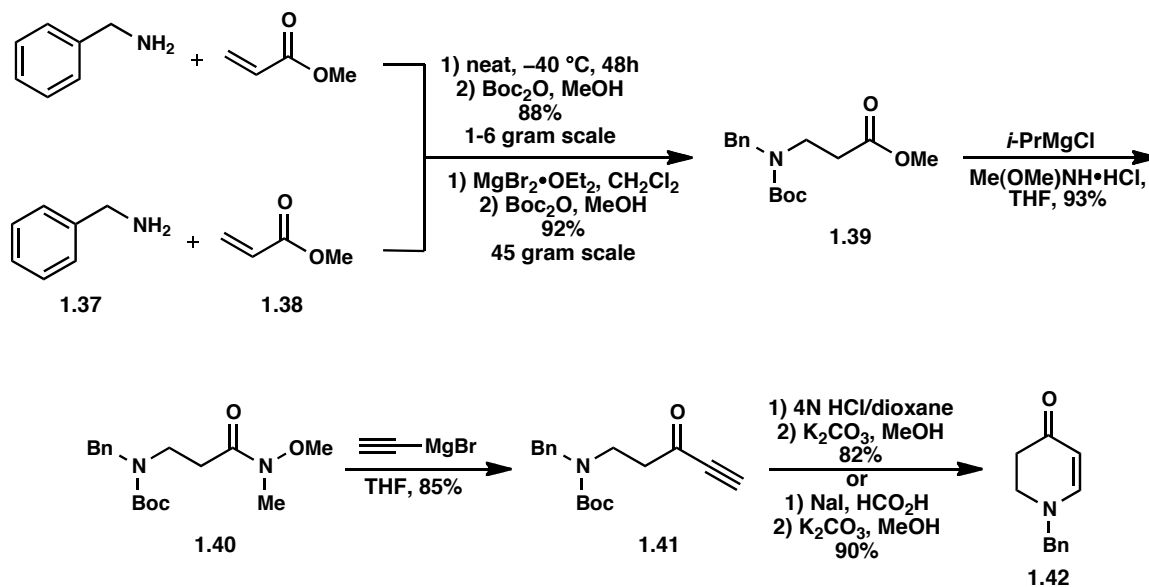


### 1.5.3 Synthesis of enaminone substrate

To test our hypothesis, we first needed to synthesize an achiral enaminone. A short concise route was devised to synthesize the 6-membered enaminone starting from benzylamine **1.37** and methyl acrylate **1.38**.<sup>47</sup> The Michael addition of benzylamine to methyl acrylate was accomplished using two different procedures, the first was run neat

while a Lewis acid catalyzed the second one. In the neat procedure, the two liquids were combined and cooled to  $-40\text{ }^{\circ}\text{C}$  for 48 h. After 48 h the reaction was warmed to room temperature and the excess benzylamine was distilled off and the resultant secondary amine was Boc protected to give  $\beta$ -amino ester **1.39** in 88% yield. This procedure was high yielding and was scalable up to 6 g, but beyond that amount the yields decreased significantly. The second procedure was superior to the first because when catalyzed by magnesium bromide ethyl etherate was complete within 24 h and performed at room temperature. Another advantage of this reaction was that it was scalable up to 45 g without a decrease in the yields.

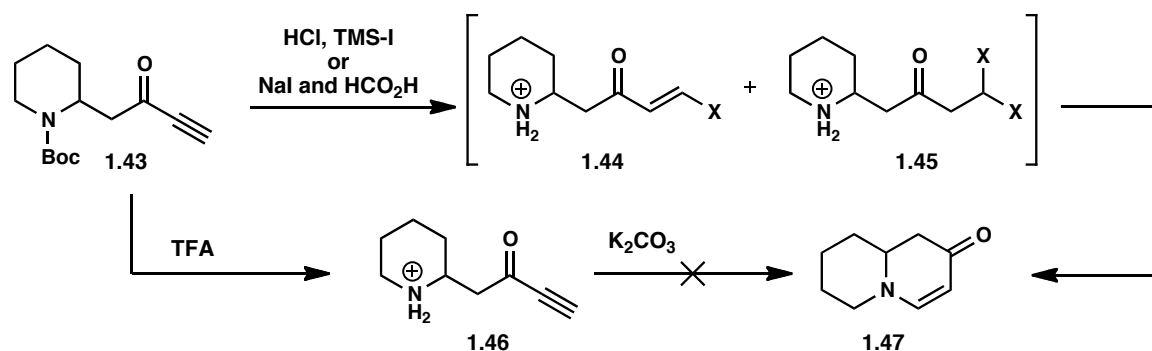
**Scheme 1-9.** Synthesis of achiral enaminone **1.42**



$\beta$ -Amino ester **1.39** was then subjected to amidation conditions following known procedures to give Weinreb amide **1.40**, which was then treated with ethynylmagnesium

bromide to furnish  $\beta$ -amino ynone **1.41**. The cyclization of  $\beta$ -amino ynones, like **1.41**, to the enaminone has been extensively studied in our group.<sup>46,47</sup> First, the Boc-deprotection of the  $\beta$ -amino-ynones can be performed by a variety of acidic conditions and the cyclization occurs under basic conditions. When studied it was uncovered that the cyclization that was disguised as a *6-endo-dig* cyclization was actually a *6-endo-trig* cyclization (Scheme 1-10). This cyclization was discovered when  $\beta$ -amino ynone **1.43** was treated with TFA to remove the Boc-protecting group and formed ammonium salt **1.46**, which would not cyclize under the basic conditions. When the Boc group was removed using HCl, TMS-I, or HCO<sub>2</sub>H with the addition of an external halide source (NaI), the desired product was formed in excellent yields.<sup>47</sup> It was surmised that the success of the reaction relies upon the transformation of the ynone to a vinyl halide (such as **1.44**) and trapping the protected amine as the ammonium salt following Boc degradation. Once the free amine is released under basic conditions, a facile 1,4-addition-elimination proceeds smoothly providing the enaminones such as **1.47**. Thus,  $\beta$ -amino ynone **1.41** was deprotected under acidic conditions with either 4N HCl/dioxane or

**Scheme 1-10.** Cyclization of  $\beta$ -amino ynones to enaminone



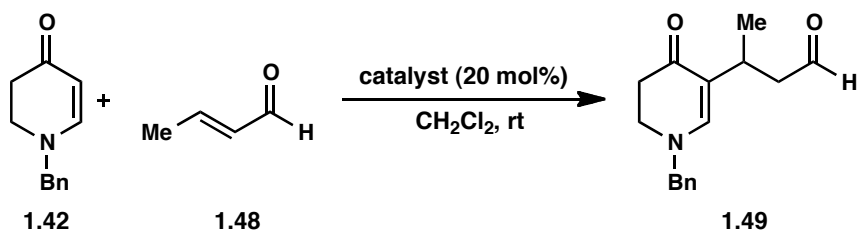


NaI and HCO<sub>2</sub>H. The resultant ammonium salt was converted to enaminone **1.42** with K<sub>2</sub>CO<sub>3</sub> and MeOH in 90% yield. The 4-step sequence was completed in a 65% overall yield.

#### 1.5.4 Proof of concept and initial scope

With enaminone **1.42** in hand, we set out to provide a proof of concept study with commercially available crotonaldehyde (Table 1-1). We initially screened three catalysts that had been shown to be useful in similar procedures.<sup>93</sup> Much to our delight the alkylation proceeded smoothly at room temperature with two of the three catalysts to form **1.49**. The initial catalyst screen showed that imidazolidinones **1.32** and **1.36** were able to catalyze the reaction, both of which were completed within four hours. No product formation was observed with the proline base catalyst **1.50** after 48 h. Given that the imidazolidinone catalysts proved to be viable catalysts for this reaction, they were chosen for further evaluation. This proof of concept study accomplished one of our goals, showing that the reaction works, but also gave us an opportunity to eventually separate the enantiomers by chiral HPLC. Going into this initial study, we knew that enantioselectivity could not be achieved at room temperature, because literature evidence suggests that lower temperatures are required.<sup>93,94</sup> However, all attempts to separate the enantiomers of **1.49** failed, forcing us to pursue other  $\alpha,\beta$ -unsaturated aldehydes.

**Table 1-1.** Proof of concept study



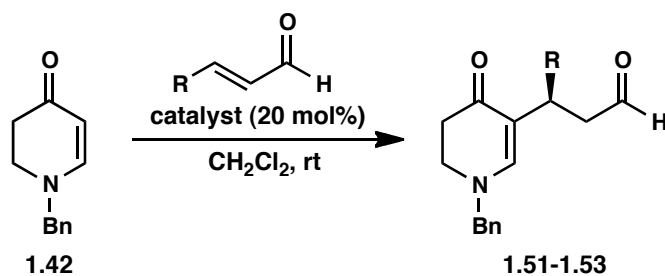
| entry <sup>a</sup> | catalyst           | yield (%) <sup>b</sup> | time (h) |
|--------------------|--------------------|------------------------|----------|
| 1                  | <p><b>1.32</b></p> | 88                     | 4        |
| 2                  | <p><b>1.36</b></p> | 76                     | 0.25     |
| 3                  | <p><b>1.50</b></p> | 0                      | 48       |

<sup>a</sup> Reaction conditions: enaminone **1.42** (1 equiv),  $\alpha,\beta$ -unsaturated aldehyde (3 equiv), organocatalyst **1.36** (0.2 equiv), in  $\text{CH}_2\text{Cl}_2$ . <sup>b</sup> Isolated yield.

With the previous results in hand, we chose to pursue three commercially available cinnamaldehydes with the expectation that the addition of an aryl moiety would help with the separation. Applying the dimethyl-imidazolidinone catalyst **1.32** to the organocatalytic reaction with the cinnamaldehydes proved to be quite fruitful (Table 1-2).

The reaction not only succeeded with cinnamaldehyde (R = phenyl, Table 1-2, entry 1), but both electron donating (OMe, Table 1-2, entry 2) and electron withdrawing (NO<sub>2</sub>, Table 1-2, entry 3) groups on the aromatic ring worked extremely well in the reaction. The aryl moiety on the product also proved to be beneficial as conditions could be worked out to separate enantiomers via chiral HPLC. As expected, very little enantioselectivity was observed with any of the products, with the best being a 1.4:1 mixture of enantiomers.

**Table 1-2.** Initial scope and enantioselectivity determination



| entry <sup>a</sup> | R | yield (%) <sup>b</sup> | time (h) | er <sup>c</sup> |         |
|--------------------|---|------------------------|----------|-----------------|---------|
| 1                  |   | 1.51                   | 84       | 4               | 1.3 : 1 |
| 2                  |   | 1.52                   | 85       | 4               | 1.4 : 1 |
| 3                  |   | 1.53                   | 81       | 4               | 1.4 : 1 |

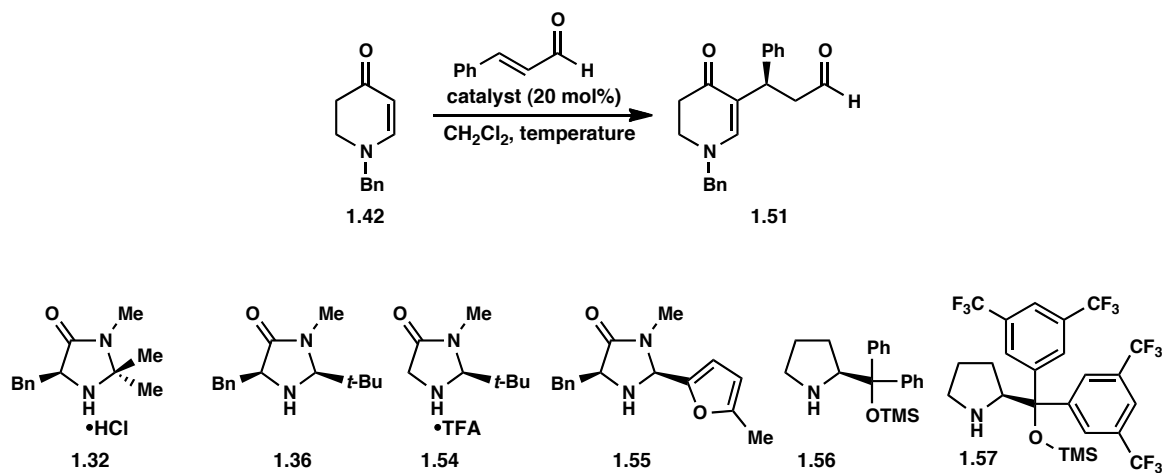
<sup>a</sup> Reaction conditions: enaminone **1.42** (1 equiv),  $\alpha,\beta$ -unsaturated aldehyde (3 equiv), organocatalyst **1.36** (0.2 equiv), *p*-TSA (0.2 equiv)<sup>b</sup> Isolated yield.  
<sup>c</sup> determined via chiral HPLC

### 1.5.5 Catalyst and temperature screen

Next, a catalyst and temperature screen was performed. Knowing that the temperature of the reaction plays a key role in the crucial interplay between reaction rate and enantioselectivity, the initial objective was to lower the temperature and monitor the enantioselectivity (Table 1-3). To optimize the catalyst and temperature, cinnamaldehyde was chosen as the electrophile because of its commercial availability. The temperature was lowered in increments of 10-15 °C and held at the desired temperature with a cryocool instrument. The temperature was initially lowered to 0 and -15 °C using dimethyl-imidazolidinone catalyst **1.32** in the reaction (Table 1-3, entries 1 and 2). Lowering the temperature to -15 °C had little effect on any aspect of the reaction (yield, reaction time or enantioselectivity). Keeping in mind that the *tert*-butyl-imidiazolidinone catalyst **1.36** was designed to increase reactivity and enantioselectivity, we employed the catalyst at the same temperatures and compared the results to the dimethyl-imidazolidinone catalyst **1.32** and found that catalyst **1.36** was superior in all aspects when compared to the dimethyl-imidazolidinone catalyst **1.32**, however the enantioselectivity was still poor at a ratio of 1.8:1 (Table 1-3, entries 3 and 4). Imidazolidinone catalyst **1.54**, which was developed to impart high stereocontrol and reactivity, gave unsatisfactory results in every aspect (Table 1-3, entries 5 and 6). Furanyl-imidazolidinone catalyst **1.55**, which has been shown to catalyze similar reactions, gave adequate yields, but the reaction times were much longer and the

enantioselectivity was worse than found with the previously investigated catalysts (Table 1-3, entries 7 and 8). Exploring several proline base catalysts gave the first indication that enantioselectivity could be improved.

**Table 1-3.** Catalyst screen



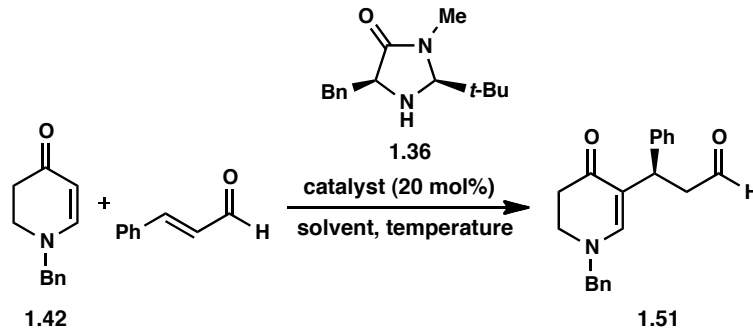
| entry <sup>a</sup> | catalyst | acid          | temp (° C) | yield (%) <sup>b</sup> | time (h) | er <sup>c</sup> |
|--------------------|----------|---------------|------------|------------------------|----------|-----------------|
| 1                  | 1.32     | HCl           | 0          | 81                     | 4        | 1.4 : 1         |
| 2                  | 1.32     | HCl           | -20        | 78                     | 6        | 1.5 : 1         |
| 3                  | 1.36     | HCl           | 0          | 79                     | 1        | 1.5 : 1         |
| 4                  | 1.36     | HCl           | -20        | 82                     | 1.5      | 1.8 : 1         |
| 5                  | 1.54     | TFA           | 0          | 41                     | 12       | 1.2 : 1         |
| 6                  | 1.54     | TFA           | -20        | 48                     | 20       | 1.4 : 1         |
| 7                  | 1.55     | <i>p</i> -TSA | 0          | 79                     | 18       | 1.2 : 1         |
| 8                  | 1.55     | <i>p</i> -TSA | -20        | 70                     | 27       | 1.6 : 1         |
| 9                  | 1.56     | <i>p</i> -TSA | 0          | 60                     | 36       | 1.5 : 1         |
| 10                 | 1.56     | <i>p</i> -TSA | -20        | 71                     | 48       | 2.3 : 1         |
| 11                 | 1.57     | <i>p</i> -TSA | 0          | 72                     | 35       | 2.5 : 1         |
| 12                 | 1.57     | <i>p</i> -TSA | -20        | 79                     | 48       | 9 : 1           |

<sup>a</sup> Reaction conditions: enaminone **1.42** (1 equiv),  $\alpha,\beta$ -unsaturated aldehyde (3 equiv), organocatalyst **1.36** (0.2 equiv), acid (0.2 equiv). <sup>b</sup> Isolated yield. <sup>c</sup> determined via chiral HPLC

Diphenyl-proline catalyst **1.56** gave good yields and better enantioselectivity than previously observed at the same temperatures (Table 1-3, entries 9 and 10). Increasing the steric demands of the proline catalyst by the addition of four trifluoromethyl groups on the phenyl ring (catalyst **1.57**) yielded much better results in terms of enantioselectivity at 9:1 (Table 1-3, entries 11 and 12). Even though the proline-based catalysts gave the best enantioselectivities at the temperatures screened thus far, the reaction times were far too long, and became even longer when the temperature was lowered. Given that literature precedent shows that *tert*-butyl imidazolidinone catalyst **1.36** imparts high enantioselectivity at temperatures below  $-20\text{ }^{\circ}\text{C}$ , it was chosen for further study.

Running the reaction at  $-78\text{ }^{\circ}\text{C}$  (Table 1.4 entry 1) and gradually letting it warm up to room temperature yielded worse results than when the reaction was held constant at  $-20\text{ }^{\circ}\text{C}$ . This suggests that the reaction is sluggish at lower temperatures and the majority of the reaction is taking place near room temperature. Decreasing the temperature to  $-40\text{ }^{\circ}\text{C}$  led to some interesting results. First, at  $-30\text{ }^{\circ}\text{C}$  the enantiomeric ratio increased slightly, however the yields dropped slightly and the reaction time increased to 36 h. At  $-40\text{ }^{\circ}\text{C}$  the enantiomeric ratios increased slightly to 2.1:1, however the reaction yield decreased significantly (42%) and the reaction time increased to 42 h. We hypothesized that the reaction yield decreased, because of the acidic co-catalyst. Since the enaminone is not stable under acidic conditions, TFA must be too strong of an acid. The longer reaction times led to increased exposure to the acid co-catalyst, eventually leading to decomposition. A literature search revealed *p*-TSA and PPTS as less acidic counterparts that have proven viable under similar reaction conditions.<sup>73</sup> Indeed, applying *p*-TSA as

the co-catalyst led to an increase in yield and a slight increase in the enantiomeric ratio in approximately the same reaction time when compared to TFA at the same reaction temperature. What can be surmised is that most likely TFA not only decomposed the starting material, but also the product. Running the reaction at  $-50\text{ }^{\circ}\text{C}$  led to an increase in the enantiomeric ratio (Table 1-4, entry 6), but the reaction time increased to 60 h. What was yet to be investigated was the addition of co-solvents. We hypothesized that the addition of a protic solvent could increase the reaction rate by increasing the turnover rate of the catalyst. This indeed turned out to be the case.

**Table 1-4.** Temperature screen

| entry <sup>a</sup> | Solvent   | acid          | temp (° C) | yield (%) <sup>b</sup> | time (h) | e.r. <sup>c</sup> |
|--------------------|---|---------------|------------|------------------------|----------|-------------------|
| 1                  | CH <sub>2</sub> Cl <sub>2</sub>                             | TFA           | -78 to rt  | 78                     | 24       | 1.5 : 1           |
| 2                  | CH <sub>2</sub> Cl <sub>2</sub>                             | TFA           | -30        | 74                     | 36       | 1.9 : 1           |
| 3                  | CH <sub>2</sub> Cl <sub>2</sub>                             | TFA           | -40        | 42                     | 42       | 2.1 : 1           |
| 4                  | CH <sub>2</sub> Cl <sub>2</sub>                             | <i>p</i> -TSA | -40        | 74                     | 45       | 2.5 : 1           |
| 5                  | CH <sub>2</sub> Cl <sub>2</sub>                             | <i>p</i> -TSA | -50        | 72                     | 60       | 10 : 1            |
| 6                  | CH <sub>2</sub> Cl <sub>2</sub> / <i>i</i> -PrOH<br>(85:15) | <i>p</i> -TSA | -50        | 73                     | 40       | 20 : 1            |
| 7                  | CH <sub>2</sub> Cl <sub>2</sub> / <i>i</i> -PrOH<br>(85:15) | PPTS          | -50        | 75                     | 48       | 15 : 1            |
| 8                  | CH <sub>2</sub> Cl <sub>2</sub> / <i>i</i> -PrOH<br>(85:15) | <i>p</i> -TSA | -78        | 73                     | 48       | 99 : 1            |

<sup>a</sup> Reaction conditions: enaminone **1.42** (1 equiv.),  $\alpha,\beta$ -unsaturated aldehyde (3 equiv.), organocatalyst **1.36** (0.2 equiv.), acid (0.2 equiv.). <sup>b</sup> Isolated yield. <sup>c</sup> determined via chiral HPLC

When the reaction was run in a mixture of CH<sub>2</sub>Cl<sub>2</sub>/*i*-PrOH (85:15) we saw a dramatic decrease in the reaction time from 60 h to 40 h (Table 1-4, entries 5 and 6). What was unexpected was the increase in enantiomeric ratio from 10:1 to 20:1 (Table 1-4, entries 5 and 6). In 2011 Brazier and coworkers, who published a systematic study on the role of protic solvents in imidazolidinone catalyzed reactions,<sup>95</sup> found that the addition of water or nucleophilic protic solvents increases the overall rate of the reaction and the enantiomeric ratio's, thereby, supporting our experimental results. PPTS as a co-catalyst not only increased the reaction times, but also led to a decrease in the enantiomeric ratio

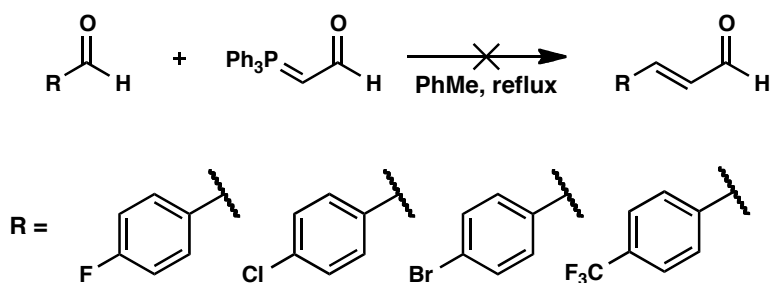


when compared to *p*-TSA (table 1-4, entries 6 and 7). Finally, lowering the temperature to  $-78\text{ }^{\circ}\text{C}$  led to a dramatic increase in the enantiomeric ratio (99:1) with no alteration of the yields and suitable reaction times (Table 1-4, entry 8).

### 1.5.6 Reaction scope

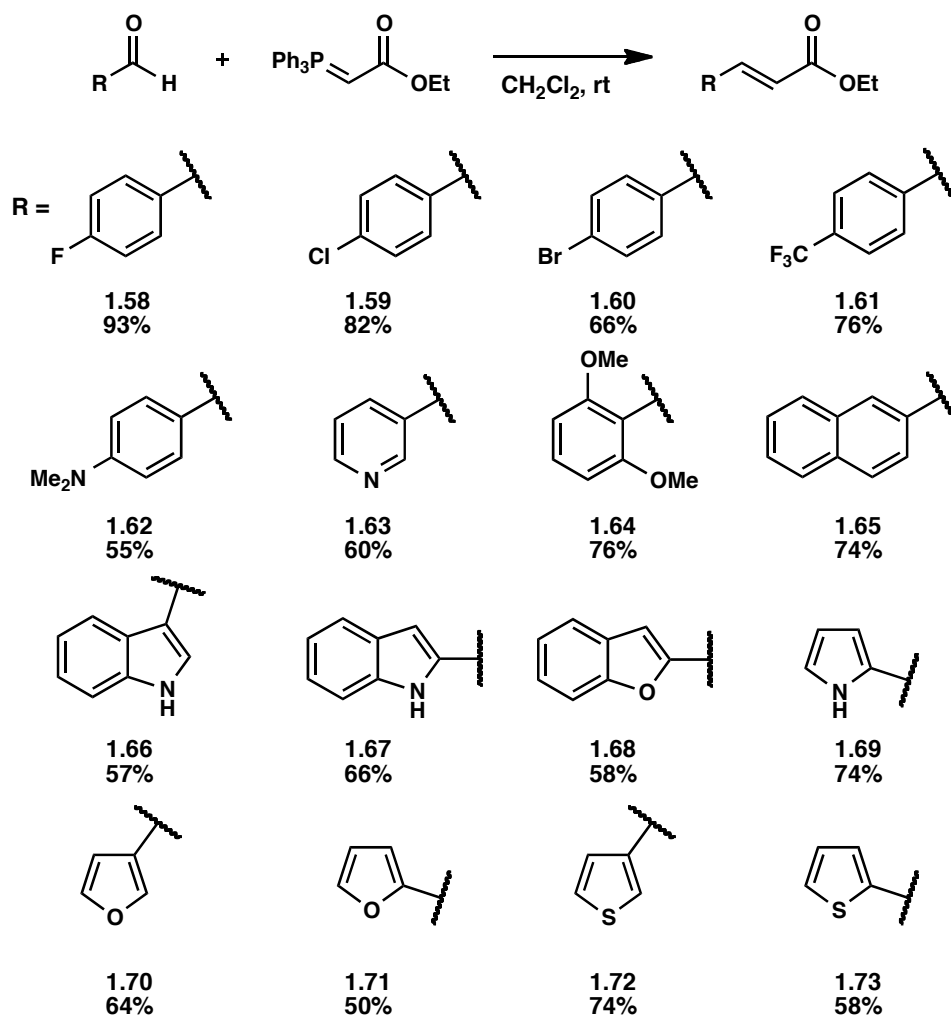
After we found suitable reaction conditions, the scope of the reaction needed to be investigated. Since we were unable to separate the enantiomers using crotonaldehyde as the electrophile, we chose to synthesize a library of cinnamaldehydes. This was first investigated using a single Wittig reaction of the appropriate aryl aldehyde with (triphenylphosphoranylidene)acetaldehyde to directly yield the  $\alpha,\beta$ -unsaturated aldehyde directly (Scheme-11). These reactions proved to be unsuccessful, presumably due to the reactivity of the product with the Wittig reagent.

**Scheme 1-11.** Direct attempts to yield  $\alpha,\beta$ -unsaturated aldehyde



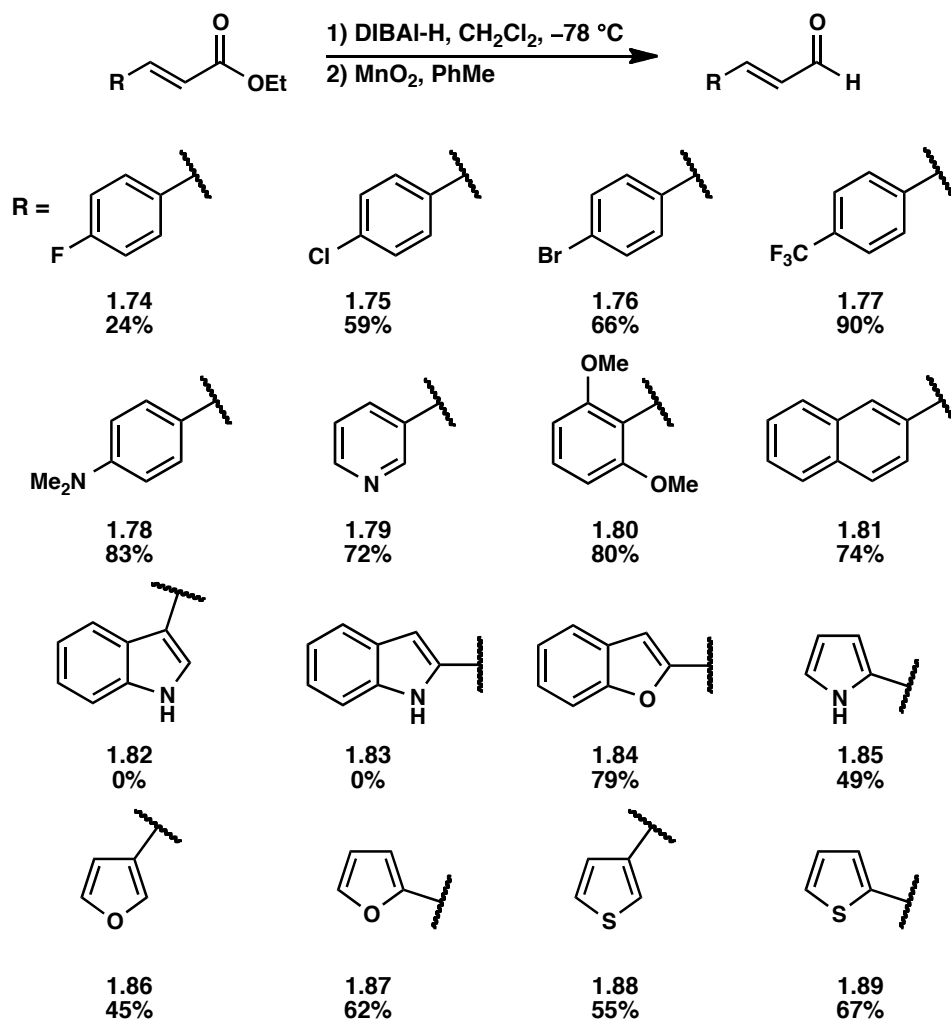
With the failure to directly synthesize the  $\alpha,\beta$ -unsaturated aldehydes, we sought out an indirect way to do so by changing the Wittig reagent. Using ethyl (triphenylphosphanyliden)acetate we synthesized a library of 16  $\alpha,\beta$ -unsaturated esters

**Scheme 1-12.** Synthesis of  $\alpha,\beta$ -unsaturated esters



with varying steric and electronic properties (Scheme 1-12). This proved to be fruitful, because the esters are much more robust and could be stored at 0 °C for >18 months with no detectable degradation by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. Next up for consideration was conversion of the oxidation state from the ester to the aldehyde. The most obvious choice was to use DIBAL-H as a reductant at  $-78$  °C. When this was performed using one equivalent of the reductant, mixtures of the aldehyde and the alcohol were obtained. To avoid this undesired mixture, an excess amount of DIBAL-H was used to reduce the ester all the way

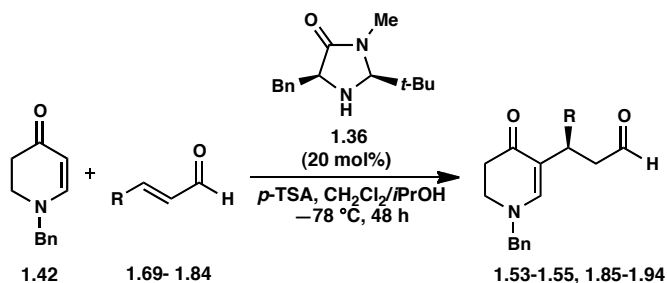
**Scheme 1-13.** Synthesis of  $\alpha,\beta$ -unsaturated aldehydes



to the alcohol. Oxidation of the allylic alcohol could be easily achieved using  $\text{MnO}_2$  (Scheme 1-13). The only drawback of the oxidation was incomplete conversion, but this could be circumvented by the addition of a large excess ( $>10$  equivalents) without overoxidation.

With a combination of commercially available  $\alpha,\beta$ -unsaturated aldehydes and the synthesized library, we set out to investigate the scope of the reaction. When electron-rich  $\alpha,\beta$ -unsaturated aldehydes were added to the enaminone high yields of the reaction products were obtained (Table 1-5, entry 2).

**Table 1-5.** Scope of  $\alpha,\beta$ -unsaturated aldehydes



| entry <sup>a</sup> | R (product) | yield (%) <sup>b</sup> | e.r. <sup>c</sup> | entry <sup>a</sup> | R (product) | yield (%) <sup>b</sup> | e.r. <sup>c</sup> |    |         |
|--------------------|-------------|------------------------|-------------------|--------------------|-------------|------------------------|-------------------|----|---------|
| 1                  |             | 1.51                   | 73                | 99 : 1             | 8           |                        | 1.94              | 64 | 73 : 27 |
| 2                  |             | 1.52                   | 81                | 95 : 5             | 9           |                        | 1.95              | 71 | 99 : 1  |
| 3                  |             | 1.53                   | 69                | 99 : 1             | 10          |                        | 1.96              | 70 | 92 : 8  |
| 4                  |             | 1.90                   | 77                | 94 : 6             | 11          |                        | 1.97              | 83 | 80 : 20 |
| 5                  |             | 1.91                   | 55                | 94 : 6             | 12          |                        | 1.98              | 74 | 83 : 17 |
| 6                  |             | 1.92                   | 42                | 99 : 1             | 13          |                        | 1.99              | 75 | 75 : 25 |
| 7                  |             | 1.93                   | 64                | 73 : 27            | 14          |                        | 1.100             | 71 | 77 : 23 |

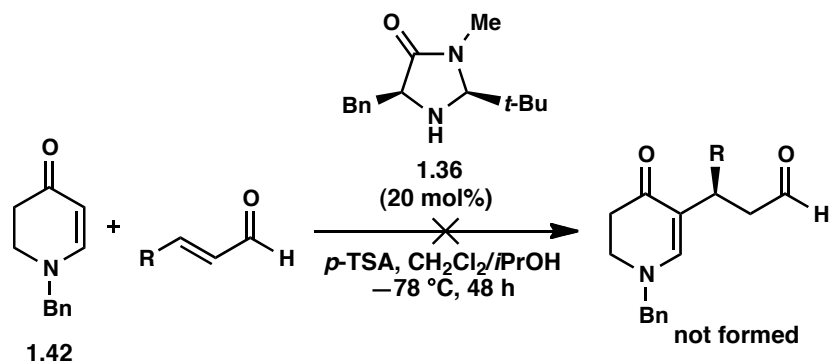
<sup>a</sup> Reaction conditions: enaminone **1.42** (1 equiv),  $\alpha,\beta$ -unsaturated aldehyde (3 equiv), organocatalyst **1.36** (0.2 equiv.),  $p\text{-TSA}$  (0.2 equiv) in  $\text{CH}_2\text{Cl}_2/\text{iPrOH}$  (85:15) at  $-78\text{ }^\circ\text{C}$ . <sup>b</sup> Isolated yield. <sup>c</sup> determined via chiral HPLC after  $\text{NaBH}_4$  reduction of the aldehyde to the alcohol

When electron-poor  $\alpha,\beta$ -unsaturated aldehydes were employed, the yields were lower, corroborating our previous results. It is noteworthy that the electronics of the aromatic ring had no bearing on the enantioselectivity of the reaction (Table 1-5, entries 2-5).

Increasing the steric hindrance at the aromatic ring decreased the yields significantly, but had no effect on the enantioselectivity (Table 1-5, entry 6). Free phenols did not disrupt the reactivity, but had a substantial negative effect on the enantioselectivity of the reaction. Reactions with heteroaromatic analogues were met with varying success. The benzofuran derivative was obtained in good yield, however the enantioselectivity decreased somewhat to 92:8 (Table 1-5, entry 10). The smaller furans and thiophenes analogues provided the reaction products in good yields but with significant lower enantioselectivities (Table 1-5, entries 11-14).

Besides giving valuable information about steric and electronic influences on the reaction, this sample set provided information about functional group compatibility. While the phenol is stable under these reaction conditions, it clearly affected the enantioselectivity of the reaction. More significantly, aryl halides are very well tolerated in the reaction, which provides a functional handle for further reactions using transition metal chemistry. The limitations of the reaction became evident when we began to investigate  $\alpha,\beta$ -unsaturated aldehydes that contained nitrogens (Table 1-6). Since the reaction is co-catalyzed by an acid, the very first event in the reaction in this case would be the protonation of the nitrogen. That leads to an inactive co-catalyst, which is a dead-end in the reaction. In theory, since the aldehyde is not the limiting reagent in the reaction (3 equivalents), the addition of excess amount of co-catalyst (3.2 equivalents) would protonate all of the nitrogens in the reaction and have enough (0.2 equivalents) left over to push the reaction forward. When this theory was examined, prolonged exposure to the acid led only to decomposition of either the starting material or the product, since both are unstable to acid.

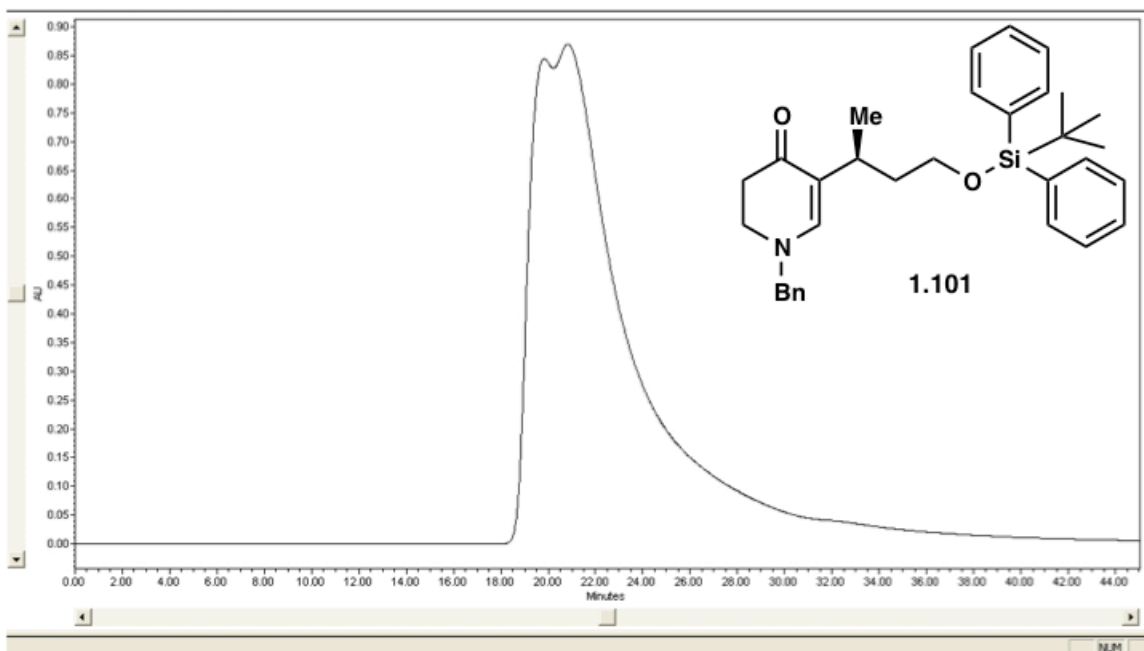
**Table 1-6.** Reaction limitations



| entry <sup>a</sup> | R (product) | entry <sup>a</sup> | R (product) | entry <sup>a</sup> | R (product) |
|--------------------|-------------|--------------------|-------------|--------------------|-------------|
| 1                  |             | 2                  |             | 3                  |             |

<sup>a</sup> Reaction conditions: enaminone **1.42** (1 equiv),  $\alpha,\beta$ -unsaturated aldehyde (3 equiv), organocatalyst **1.36** (0.2 equiv), *p*-TSA (3.2 equiv) in CH<sub>2</sub>Cl<sub>2</sub>/*i*PrOH (85:15) at -78 °C.

After obtaining promising results with the cinnamaldehyde type electrophiles, we renewed our interest in finding conditions to separate the enantiomers formed in the reaction with alkyl substituted  $\alpha,\beta$ -unsaturated aldehyde (crotonaldehyde). In both cases (cinnamaldehyde and alkyl  $\alpha,\beta$ -unsaturated aldehydes) the aldehydes were reduced to the alcohol before separation by chiral column chromatography. The first strategy was to modify the alcohol with lipophilic groups to aid in

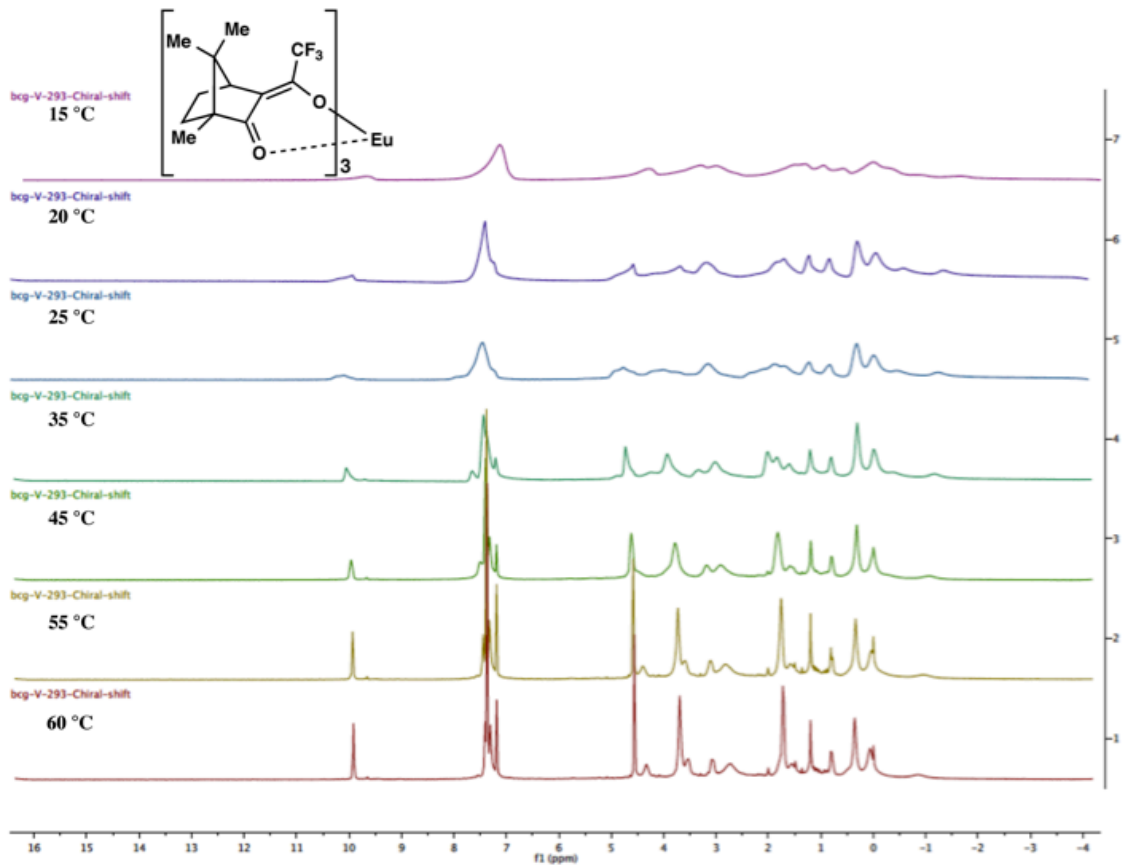


**Figure 1-10.** Separation of alcohol **1.101** by chiral column chromatography.

the separation. All of the modifications that we carried out with the alcohol (benzyl, *p*-OMe-benzyl, trityl, and TBDPS) proved to be unsuccessful. In fact, the best separation via chiral HPLC was achieved using the TBDPS protecting group, but the separation left much to be desired, to say the least (Figure 1-10).

We next turned our attention to NMR techniques. The easiest way to approach this was to use lanthanide based chiral shift reagents. This seemed plausible, because we had copious amounts of aldehyde **1.49** on hand from the previous derivatization studies. We first prepared a 10 mM stock solution of the chiral shift reagent in  $\text{CDCl}_3$  and added small aliquots of this solution to the aldehyde, and monitored the differences by  $^1\text{H}$  NMR. The mixture was heated to 60 °C in increments. With the addition of 50  $\mu\text{L}$  of the chiral shift reagent (10 mM solution in  $\text{CDCl}_3$ ) we noticed that upon heating the mixture;

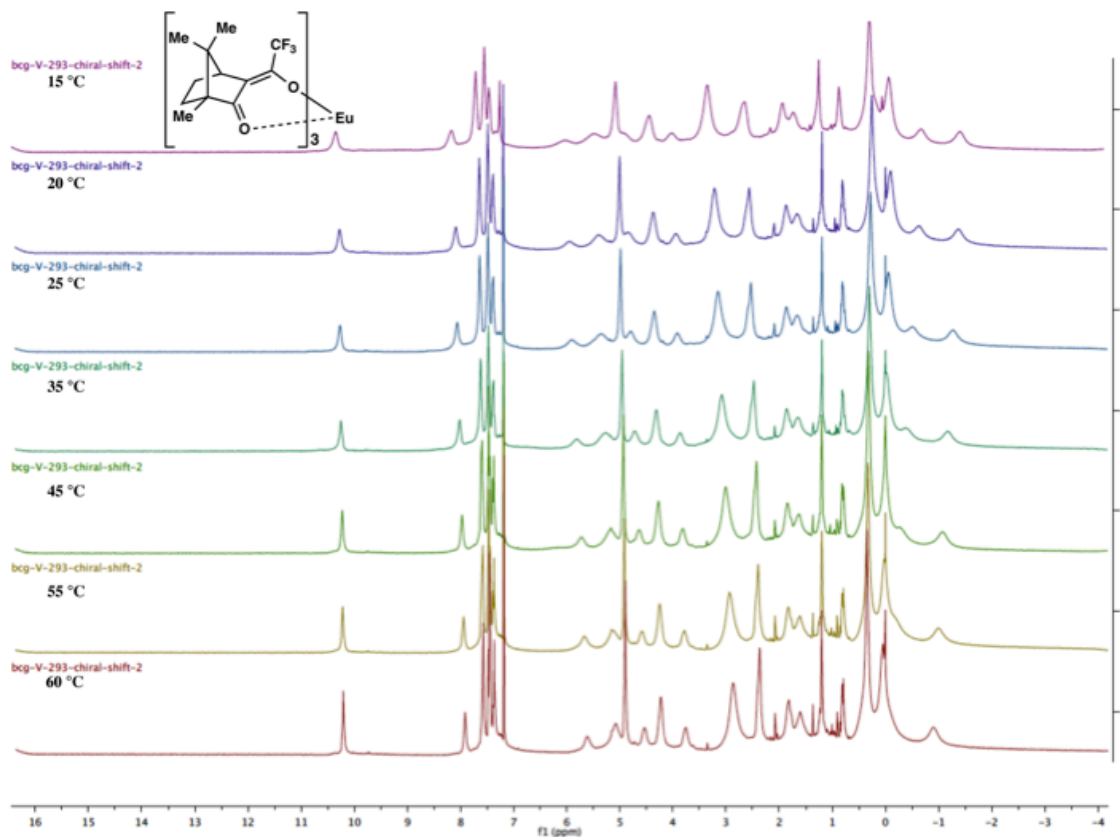




**Figure 1-11.**  $^1\text{H}$  NMR study **1.49** with 50  $\mu\text{L}$  of chiral shift reagent added.

the resolution of the spectra improved (Figure 1-11). However, nothing discernable is evident from the spectra. Since we are trying to separate the racemic mixture, we should see roughly a 1:1 mixture of diastereomeric peaks, which is not seen with the addition of 50  $\mu\text{L}$  of the chiral shift reagent. Another 50  $\mu\text{L}$  of the chemical shift reagent was added (100  $\mu\text{L}$  total) and the experiment was repeated (Figure 1-12.). Again, we noticed that as the mixture is heated up, the spectral resolution increases. We turned to 2D HMQC NMR

analysis to see if any proton resonances correlated to the same carbon. All attempts at establishing a correlation between diastereomeric protons proved to be unsuccessful.

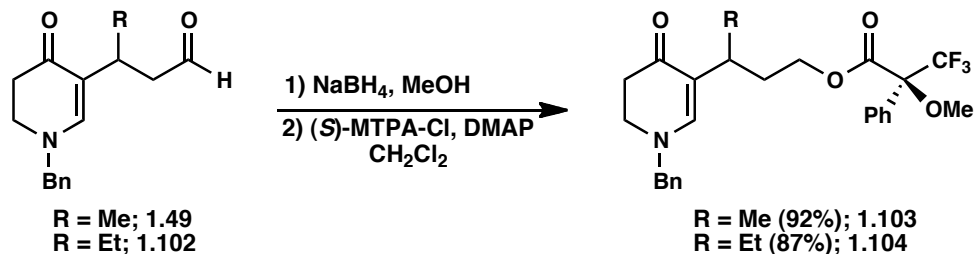


**Figure 1-12.**  $^1\text{H}$  NMR study **1.49** with 100  $\mu\text{L}$  of chiral shift reagent added.

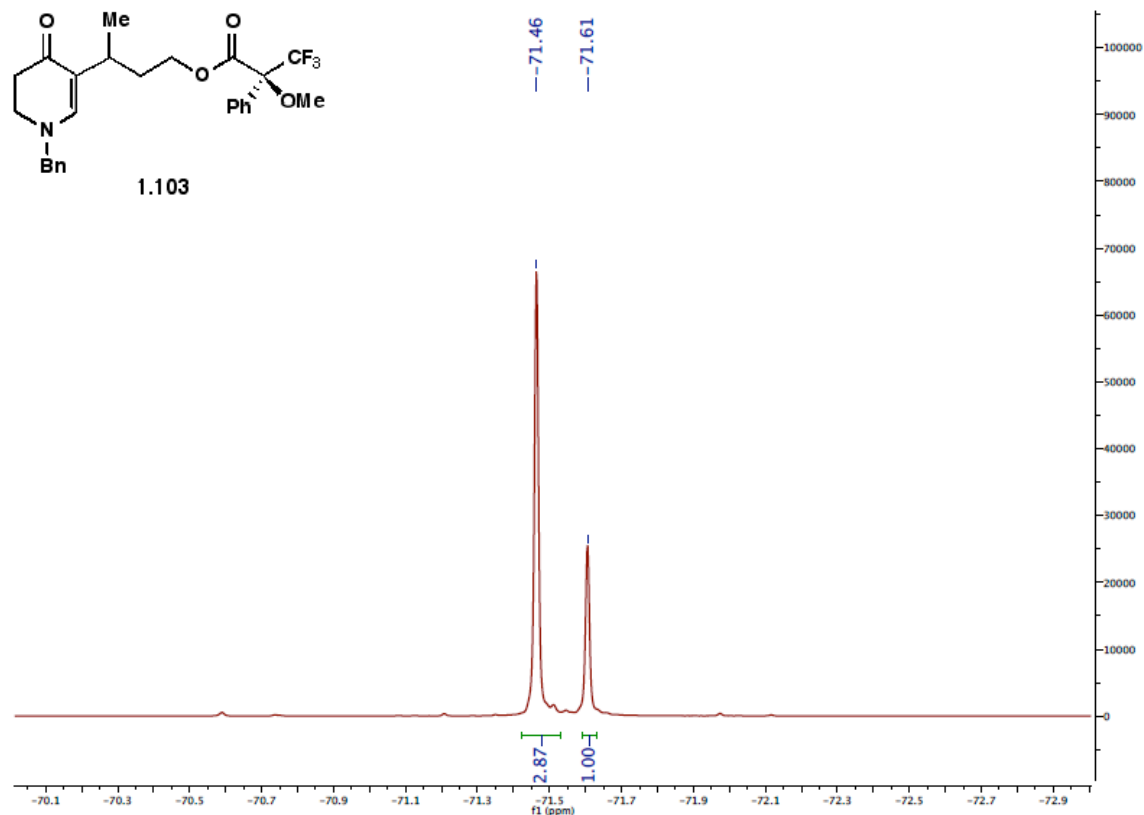
We next decided to make use of the Mosher ester technique and prepare fluorine-containing diastereomers. There are inherent advantages to fluorine NMR. Fluorine has only one naturally occurring isotope,  $^{19}\text{F}$ , and is an ideal nucleus for study by NMR. Furthermore, because of its high abundance, NMR measurements are fast and the integrals are as reliable as  $^1\text{H}$  resonances.<sup>96</sup> When a racemic alcohol is reacted with a non-racemic Mosher's acid chloride, only two diastereomers can result from the reaction, and

the diastereomeric ratio would be equivalent to the enantiomeric ratio archived in the reaction.

**Scheme 1-13.** Synthesis of Mosher esters



To this end, we set out to synthesize esters **1.103** and **1.104** (Scheme 1-13). Aldehydes **1.49** and **1.102** were reduced with  $\text{NaBH}_4$  at  $-15\text{ }^\circ\text{C}$ , which furnished the corresponding alcohols. Next, the alcohols were subjected to reaction with *(S)*-MTPA-Cl and DMAP to yield esters **1.103** and **1.104**.<sup>97</sup> Once the esters were on hand we performed  $^{19}\text{F}$  NMR analysis. It was immediately obvious that this method furnished two diastereomers and that the two peaks were present in approximately a 1:1 ratio. Applying this method to the products **1.97** and **1.98** we observed approximately a 3:1 ratio of diastereoisomers ( $^{19}\text{F}$  NMR shown in Figure 1-13 for **1.97**). This is not entirely surprising given the fact the smaller heterocyclic cinnamaldehyde derivatives gave approximately the same enantiomeric ratios.



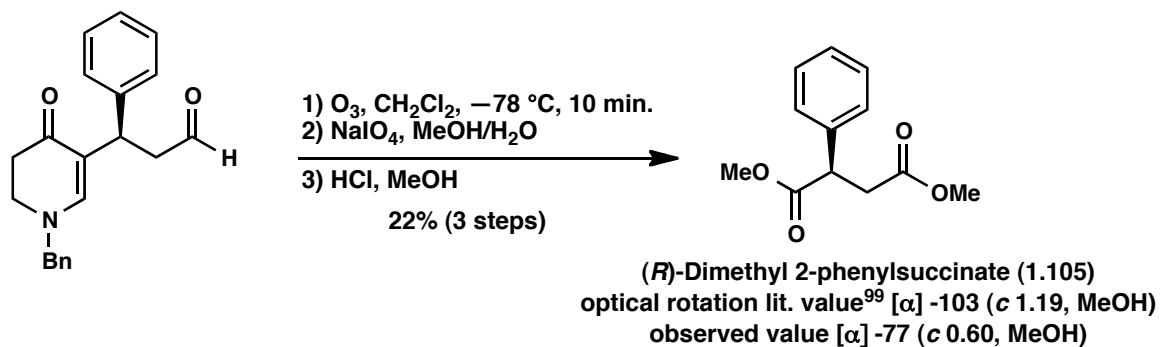
**Figure 1-13.**  $^{19}\text{F}$  NMR of diastereomeric Mosher esters 1.103.

## 1.6 Determination of absolute stereochemistry

In order to determine the stereochemical outcome of the reaction, we first tried to crystallize the product **1.90** of the organocatalytic reaction containing the 4-bromophenyl substituent. Several different techniques (slow evaporation, slow diffusion, and solvent diffusion) were unsuccessful. The hydrazone was also synthesized and the same crystallization techniques were applied, however they were also unsuccessful. Analyzing the general product structure, we hypothesized that we could obtain a dicarboxylic acid in relatively short order. This could be extremely useful because the degradation product

could be transformed into a known compound. To test this hypothesis we subjected organocatalytic product **1.51** to ozonolysis conditions (Scheme 1-14). The resultant diketone was oxidatively cleaved using an excess of sodium periodate<sup>98</sup> along with concomitant oxidation of the aldehyde resulted in the phenyl succinic acid, a known compound. This acid proved to be very difficult to purify. To circumvent this the crude acid was subjected to Fischer esterification conditions with HCl/MeOH to yield the diester, also a known compound.<sup>99</sup> The optical rotation of the diester was compared to literature values thereby confirming the absolute stereochemistry of the products.

**Scheme 1-14.** Proof of absolute stereochemistry



## 1.7 Summary

In summary, our methodology provides a direct route for the construction of  $\alpha$ -alkylated enaminones. Although the substrate scope is large in terms of yield, the scope in terms of enantioselectivity is currently limited to cinnamaldehyde type electrophiles. This method provides a significant advancement in which  $\alpha$ -alkylation was previously notorious for being very difficult, and certainly not enantioselective.

## Chapter 2

### Phenanthropiperidine Natural Product Analogs as Potential Anti-Cancer Agents

#### 2.1 Introduction to Phenanthropiperidine Alkaloids

Historically, natural products have been an invaluable resource for the discovery of bioactive natural products and that continues to be the case.<sup>100,101</sup> However, the discovery of bioactive natural products is just the beginning in the pursuit of drug discovery. Natural products can be viewed as an advanced lead, but still may require considerable optimization before they can be considered for potential therapeutic use. The phenanthropiperidines are a great example of this theory. These alkaloids have been pursued as leads for the treatment of cancer and immune-related diseases for over half a century, yet not a single member has emerged for use in the clinic. The previous statement explains why some have lost interest in these alkaloids, while others continue the search for innovative solutions to make this class clinically relevant. The reasons for both will be discussed below.

The phenanthropiperidines were isolated from plants that have been used in folk medicine for centuries.<sup>102</sup> The therapeutic reputation of the plant *Tylophora indica* peaked the interest of researchers to try and validate, as well as characterize the component/s for biological effects. The phenanthropiperidines have been known for over sixty years and now are gaining more attention than ever. The renewed interest is explained by advances in biotechnology that are uncovering the intricate mechanism of action/s induced by these alkaloids. This introduction provides a review of the chemistry and biology of the phenanthropiperidines, starting with the discovery of tylophorine in 1935.<sup>103</sup> Unlike other

reviews<sup>104-107</sup> my review is not limited to the most recent literature, but rather will consolidate the widely dispersed literature with special attention given to the pharmacologically relevant details in order to better understand the mechanism/s of action. Given that the accessibility of a natural product (i.e., isolation or synthetic means) is a major concern for natural product drug discovery; the preparation of these alkaloids will also be covered.

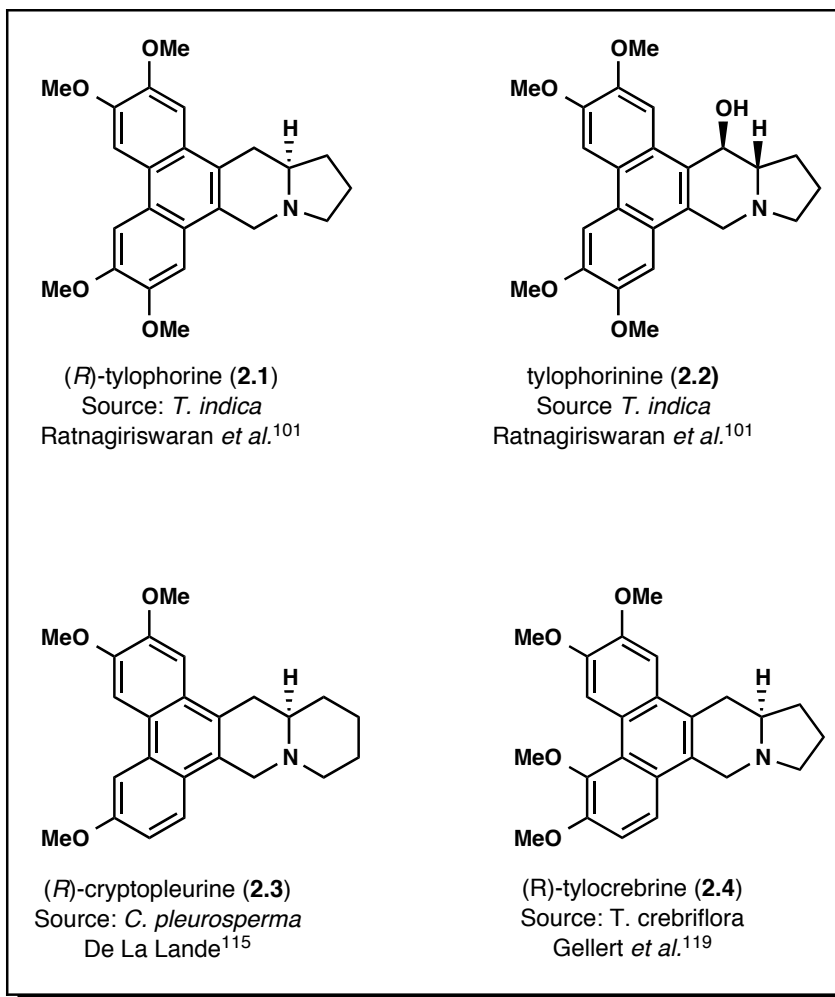
### 2.1.1 Early Phenanthropiperidines

Folk medicines are a rich source of incipient drugs that are laden with clues for therapeutic use. In the late 19<sup>th</sup> century scientists discovered that *T. indica* was used as an Indian household remedy for a broad spectrum of medical conditions.<sup>108</sup> In 1935, Ratnagiriswaran *et al.* extracted and purified two alkaloids from the perennial climbing plant, naming the major component tylophorine (**2.1**) and the minor component tylophorinine (**2.2**) as shown in Figure 2-1.<sup>103</sup> However, the structure of tylophorine was not determined until 1960 through degradation studies and comparison with synthetically derived material.<sup>109-114</sup> The proposed structure contained an indolizidine fused to a symmetrically substituted phenanthrene.<sup>113</sup> The originally mistakenly assigned stereochemistry<sup>115</sup> was unambiguously reassigned as *R* following a total synthesis, which enabled a comparison of optical rotations.<sup>116</sup>

Although tylophorine was the first phenanthropiperidine to be isolated, another member of its class was isolated and structurally characterized several years before tylophorine's structure was determined. In 1948 De la Lande reported the isolation of cryptopleurine (**2.3**), a highly toxic substance extracted from a walnut tree indigenous to



Australia called *Cryptocarya pleurosperma*.<sup>117</sup> Then, in 1954 the structure of cryptopleurine was determined through X-ray diffraction studies of its methiodide



**Figure 2-1.** Structure of early phenanthropiperidines.

congener.<sup>118-120</sup> The very first pharmacological reports revealed that cryptopleurine was lethal in rat, mouse, rabbit, dog, and cat models even at very low doses (1.5 to 5 mg/kg). It also became evident that the alkaloid was an irritant, because upon oral administration

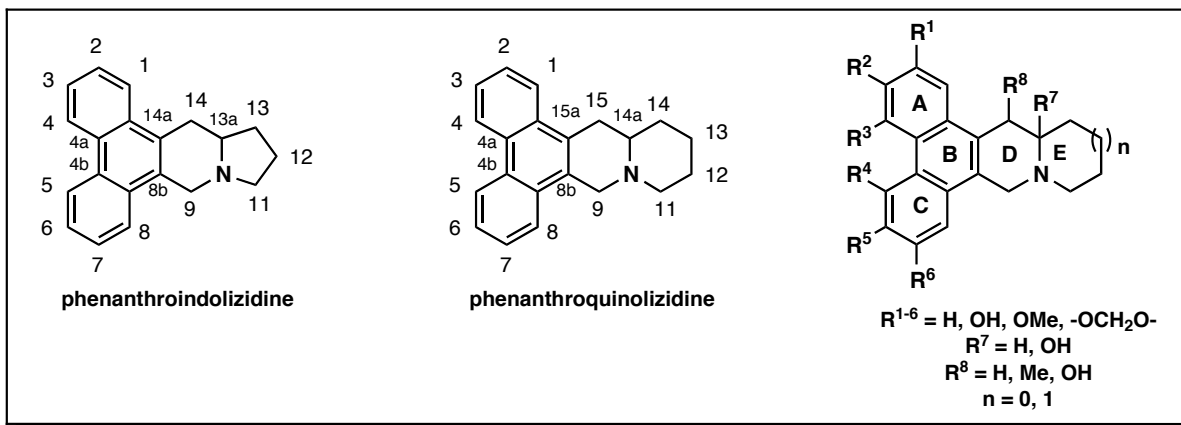
the rats developed blisters on their face and paws. Additionally, topical administration in humans produced vesication on the arm and face that lasted several weeks and ulcerations were found in the gastrointestinal tract of rabbits that had received oral doses.

The fourth member of the class is tylocrebrine (**2.4**), and probably the most infamous. Tylocrebrine was isolated in 1962 from a vine in North Queensland, *T. crebriflora*, which had vesicant properties.<sup>121</sup> Tylocrebrine's structure was found to be a regioisomer of tylophorine. Because of the evidence that the phenanthropiperidines had anti-cancer properties, tylocrebrine was tested and found to be more effective than cryptopleurine, tylophorine, and tylophorinine in several cancer models.<sup>122</sup> Only three years after being isolated, tylocrebrine entered a clinical trial.<sup>104</sup> Unfortunately, the trial was discontinued the same year, before the therapeutic potential was established. Suffness and Cordell reported the trial was discontinued due to adverse neurological side effects.<sup>104</sup> Because of this result from the clinic, interest in the alkaloids declined in the years to follow. Cryptopleurine was an indiscriminating poison, tylophorine and tylophorinine had only demonstrated mediocre anti-cancer activity, and tylocrebrine was potentially neurotoxic.

### **2.1.2 Phenanthropiperidine Isolation and Structure**

Since 1965 over 60 phenanthropiperidines and closely related analogs have been isolated and characterized. They are primarily found in two plant families, the Asclepiadaceae and Moraceae.<sup>107</sup> Five genera (*Tylophora*, *Cynanchum*, *Vincetoxicum*, *Pergularia*, and *Antitoxicum*) out of the 300 genera are known to contain these alkaloids as compared to only one genus (*Ficus*) of the Moraceae family. These plants are found

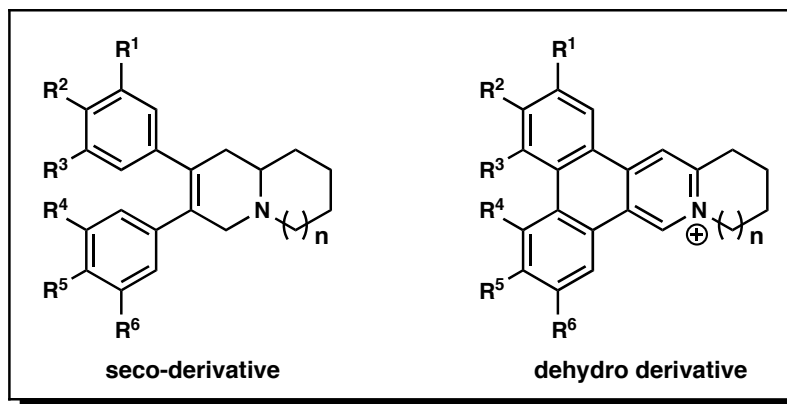
throughout Asia, Africa, Australia, and the South Pacific. Fortunately there are a few reviews on this subject.<sup>104,106</sup>



**Figure 2-2.** General structural features and numbering of phenanthropiperidines.

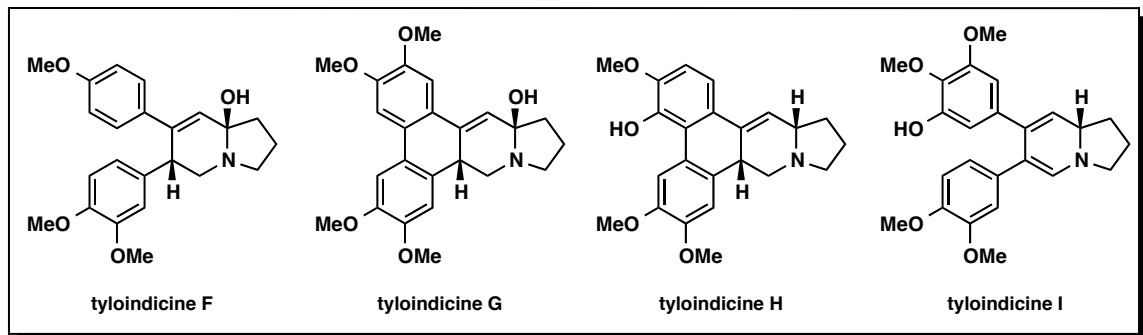
The general structural features of these alkaloids are a bicyclic nitrogenous heterocycle and a polyoxygenated phenanthrene system. The phenanthrene system is generally substituted with three to five methoxy or hydroxy groups, although methylenedioxy substituents have been isolated as well.<sup>123,124</sup> A pyrrolidine or a piperidine make up the terminal aliphatic heterocycle (ring E), as denoted by the names phenanthroindolizidine and phenanthroquinolizidine. (4a,4b)-Seco- and dehydro-derivatives, where the D ring is fully aromatized, are frequently isolated along with the phenanthropiperidine parent compounds. This isolation of these compounds show the full spectrum of oxidation states that exist at any given time within the plant.<sup>105,106</sup> Oxygen can also be introduced at the C14/15 in the indolizidines and quinolizidines as well as the nitrogen forming phenanthropiperidine *N*-oxides.<sup>125</sup> Johns *et al.* isolated a rare phenanthroquinolizidine alkaloid cryptopleuridine, which possesses a C12-hydroxyl

group.<sup>126</sup> To date, this is the only known natural phenanthropiperidine that has a functionalized E ring.



**Figure 2-3.** Phenanthropiperidine oxidation states.

In 1984, another structural anomaly was observed in the alkaloids of cultivated *T. hirsta*.<sup>127,128</sup> These natural product alkaloids were essentially identical to several phenanthropiperidines already isolated except they had a 13a-methyl substitution ( $R^8 = \text{Me}$ ). Pettit and coworkers isolated and characterized several more of these alkaloids from *Hypoestes verticillaris*.<sup>129</sup> Interestingly, in wild populations of *T. hirsta*, the primary alkaloidal constituent bears a hydroxyl group at the bridgehead ( $R^8 = \text{OH}$ ) instead of the methyl functionality.<sup>130</sup> This labile hemiaminal functional group has since been reported in three tyloindicines isolated in 1991 by Ali and coworkers (Figure 2-4).<sup>131</sup> Besides the rare 13a-hydroxy group that appears in tyloindicine F, G, and H, these members also have a precariously positioned olefin that is out of conjugation with ring C.



**Figure 2-4.** Unusual structures of the tyloindicines.

Tyloindicine I is also unique in this respect. However, the structures of tyloindicines F and G are met with some skepticism.<sup>105</sup> The stereochemistry of the hydroxy group in the hemiaminal of the tyloindicines F and G was proposed based solely on optical rotation and needs yet to be verified.

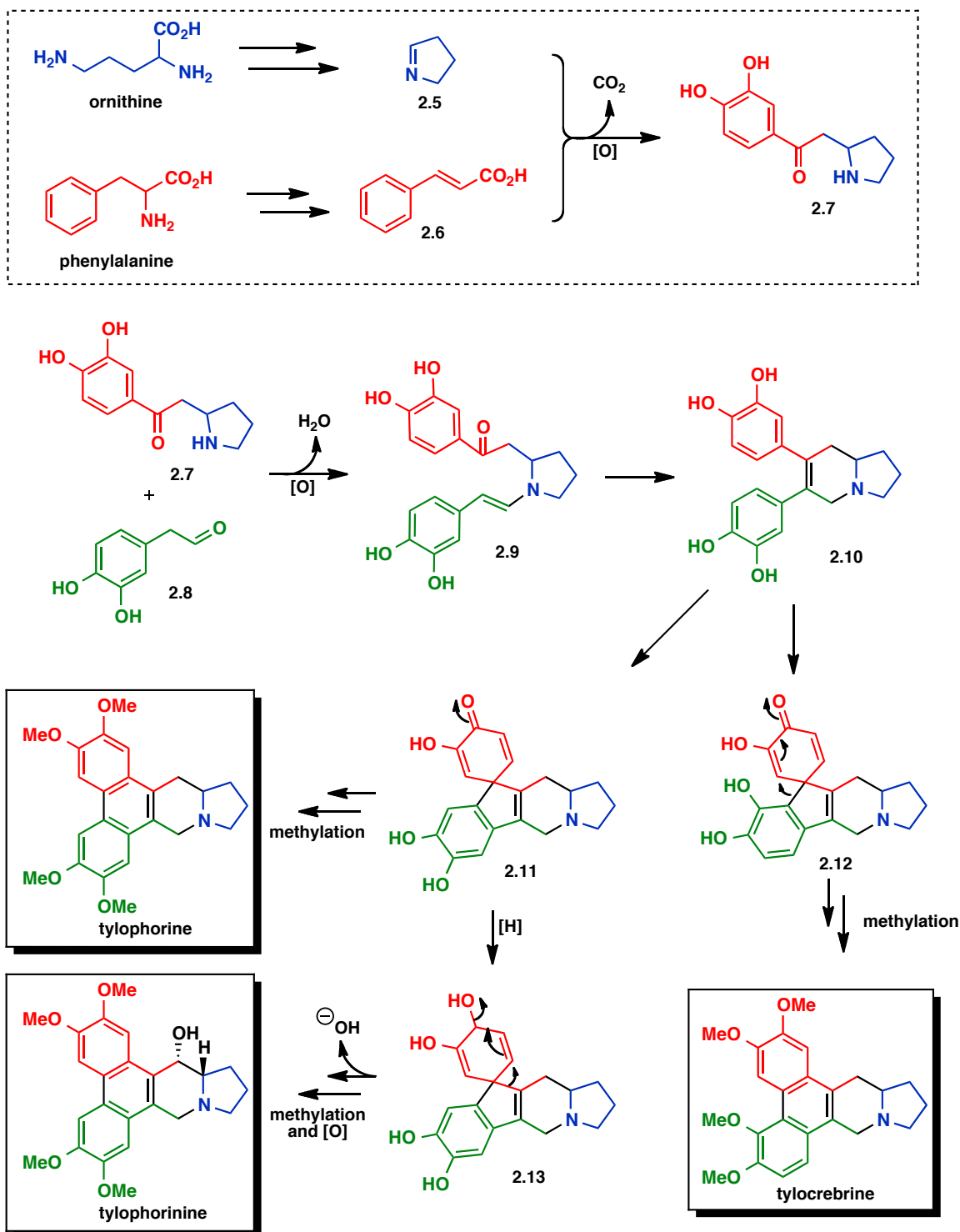
The variety of structures that are found within this class has aided an understanding into the structure-activity relationships (SAR) and provided many challenging targets for synthetic organic chemists. There have been a few reviews that have covered the various strategies used for synthesizing these molecules and will be discussed later on.<sup>105,106</sup> It is important to note that total syntheses of these alkaloids have provided a sustainable source and have largely supplanted isolation efforts.

### 2.1.3 Biosynthesis

Although the biosynthesis of the phenanthroindolizidines is better understood than that of the phenanthroquinolizidines, it is likely that the pathways are analogous.<sup>132</sup> A representative biosynthetic pathway is outlined in Scheme 2-1. It should be noted that the extent of phenolic methylation of each intermediate is uncertain and is most likely

liberally regulated as evidenced by the diversity seen in the natural products, notwithstanding the representative structures in Scheme 2-1. These pentacyclic alkaloids are thought to be derived from tyrosine (Tyr),<sup>133</sup> phenylalanine (Phe),<sup>134</sup> and ornithine.<sup>133</sup> The phenanthroquinolizidine biosynthesis varies only with respect to the latter precursor, which is derived from lysine instead of ornithine. In the indolizidine class, the pyrrolidine carbons originate from ornithine, which undergoes a decarboxylation and subsequently deaminates and cyclizes to yield 3,4-dihydro-2*H*-pyrrole (**2.5**). This cyclic imine further reacts with cinnamic acid **2.6**,<sup>135</sup> synthesized from Phe affording a carboxylic acid intermediate (not shown). Following decarboxylation and alcohol oxidation amine **2.7** undergoes condensation with 3,4-dihydroxyphenylacetaldehyde **2.8** which is derived from Tyr.<sup>136</sup> The resultant enamine **2.9** cyclizes, and upon dehydration yields the seco-derivative **2.10**. As already discussed, isolation of the seco-derivatives is common, which gives credence to this biosynthetic pathway. The final transformation is thought to proceed through an oxidative coupling and subsequent rearrangement,<sup>135</sup> but to date there is no direct evidence that supports this hypothesis.

**Scheme 2-1.** Proposed biosynthesis of phenanthropiperidines



Mulchandani *et al.* suggested that this process is initiated with the formation of dienones **2.11** or **2.12** through *ortho*- or *para*-phenolic couplings. The strained spirocycles then undergo a 1,2-alkyl shift to complete the biosynthetic sequence of tylophorine or tylocrebrine. Another fate for spirocycle **2.11** was also proposed to explain the formation of tylophorinine. In this case, dienone **2.11** is reduced to dienol **2.13** prior to rearrangement resulting in the elimination of hydroxide upon phenanthrene formation. Despite the ambiguities of this sequence, this mechanistic hypothesis is plausible when considering its resemblance to the phenolic couplings seen in the biosynthesis of morphine.<sup>137</sup> Moreover, this hypothesis has considerable explanatory power, needing minimal revision to explain the biogenetic origins of multiple naturally occurring phenanthropiperidines.

#### **2.1.4 Biology of *T. indica***

*Tylophora* extracts have gained considerable attention for the treatment of inflammatory diseases and, to a much lesser extent, cancer. Chitnis *et al.* investigated the cytotoxic properties of leaf and plant extract of *T. indica* in mice presenting transplantable leukemia tumors (either L1210 or P388).<sup>138</sup> Notably, mice receiving daily doses of extract survived longer than those treated with a placebo. Since the anti-cancer properties of *T. indica* have been attributed primarily to specific phenanthropiperidine alkaloids, most investigations have focused on the pharmacology of the purified alkaloids rather than on plant-derived mixtures and hence will be discussed later. On the other



hand, the efficacy and mode of action of *T. indica* for the treatment of immune disorders, such as asthma, have been extensively investigated.<sup>139</sup>

#### 2.1.4.1 Clinical studies

Six clinical studies have been conducted using the extracts of *T. indica* for the treatment of asthma (Table 2–1). Shivpuri *et al.* conducted two double blind clinical investigations of *T. indica*.<sup>140,141</sup> In these studies, the leaves of *T. indica* were administered to over 100 patients with asthma and allergic rhinitis. After a six-day regimen, patients experienced significant symptomatic relief that lasted for several weeks. In a follow-up study, ethanolic extracts were administered to 195 asthmatics.<sup>142</sup> Over 50% experienced moderate to complete symptomatic cessation compared with 31% of patients taking a placebo. Four other clinical investigations have been conducted with either extracts or powdered plant material.<sup>143-145</sup> Two studies showed appreciable symptomatic remission,

**Table 2-1.** Clinical studies with *T. indica* for the treatment of asthma

| source            | # of participants | study length | results   | reference |
|-------------------|-------------------|--------------|---|-----------|
| leaves            | 166               | 6 days       | 50-100% symptomatic relief  | 136       |
| leaves            | 110               | 12 weeks     | 62% vs 28% (control) improvement at 6 days;<br>16% improvement at 12 weeks  | 135       |
| EtOH extract      | 195               | 12 weeks     | 50% of participants experienced symptomatic relief<br>vs. 31% of placebo    | 137       |
| alkaloid extract  | 123               | 12 weeks     | Improvement in symptomatic relief and FEV<br>effects peak at 4 weeks        | 140       |
| powdered plant    | 135               | 3 weeks      | no significant improvement of symptoms                                      | 138       |
| dried leaf powder | 30                | 16 days      | nocturnal dyspnea symptoms improved;<br>sustained rise in MBC, VC, and PEFR | 139       |
| dried leaf powder | 29                | 4 weeks      | decreased eosinophile count;<br>increased 17-ketosteroid secretion          | 141       |

FEV1 = forced expiratory volume (in 1 second); MBC = maximum breathing capacity; VC = vital capacity; PEFR = peak expiratory flow rate.

while the other reported no statistically significant difference between patients receiving the treatment and placebo. In pursuit of a physiological explanation for the apparent symptomatic relief, Gore *et al.* demonstrated that dried leaf powder had immunosuppressive properties.<sup>146</sup> Interestingly, the immunosuppression, as judged by an eosinophil count, coincided with elevated 17-ketosteroid secretion, suggesting an indirect effect through stimulation of the adrenal cortex. Notably, increased levels of eosinophils in the sputum of asthmatics have a strong correlation with bronchial inflammation.<sup>147</sup>

An assessment of the methodologies used in five of these clinical trials has recently been reported.<sup>148</sup> Although the positive effects of *T. indica* are clear, the authors conclude that the clinical relevance of several of these studies was undermined by poorly designed experiments or the omission of important details (age range of participants, dropouts/withdrawals etc...). Considering the prevalence of asthma and the appeal of complimentary medicine, there is a need for further characterization of the efficacy and safety of *T. indica*,<sup>149</sup> which is currently marketed in products such as AsmaAide™, Ultra Asthmatica, and T. Asthmatica Plus.

#### **2.1.4.2 *In vitro* and *in vivo* studies**

Preclinical studies have been instrumental in uncovering the details of the anti-asthmatic effects of *Tylophora* extracts. A summary of these investigations is shown in Table 2-2. Haranath and Shyamalakumari studied the effect of *T. indica* on *in vivo* immunosuppression.<sup>150</sup> Aqueous extracts administered intraperitoneally prevented anaphylaxis in guinea-pigs 10 days after its administration and caused leucocytosis and leucopenia—indicative signs of immunosuppression—in dogs upon *i.v.* administration.

Atal *et al.* later reported the immunomodulating properties of ethanolic extracts of *T. indica*.<sup>151</sup> Significant inhibition of the humoral immune response (HIR), stimulation of phagocytic function, and a significant increase in survival time for a foreign skin graft were reported. Adrenergic effects were noted by Udupa *et al.*<sup>152</sup> Upon treatment of male albino rats with the extracts of *T. indica*, the adrenals increased in weight and decreased in cholesterol and vitamin C content indicating direct stimulation of the adrenal cortex.

**Table 2-2.** *In vivo* and *in vitro* studies with *T. indica* extracts

| extract    | route/dose                             | model                               | result  | reference |
|------------|--|-------------------------------------|---|-----------|
| aqueous    | <i>i.p.</i> , <i>i.v.</i>              | guniea pig<br>dog                   | prevented anaphylaxis<br>leucosytosis and leucopenia                                | 145       |
| ethanolic  | oral                                   | mouse                               | ↓ DHT<br>↓ HIR<br>↑ survival time in skin allograft rejection                       | 146       |
| multiple   | oral, <i>i.p.</i>                      | rat                                 | stimulation of adrenal cortex   | 147       |
| alkaloidal | oral                                   | rat, mouse                          | ↓ DTH<br>↓ DNFB-contact sensitivity<br>↓ IgM response                               | 149       |
| alkaloidal | >300 pg/mL<br>19-300 pg/mL<br>39 ng/mL | T cells<br>macrophage<br>macrophage | ↓ IL-2, ↓ proliferation<br>↑ IL-2, ↑ proliferation<br>↑ IL-1, macrophage activation | 148       |

*i.p.* = intraperitoneal; *i.v.* = intravenous; DTH = Delayed-type hypersensitivity; HIR = Humoral immune response; DNFB = dinitrofluorobenzene.

With evidence that *T. indica* can be used for the treatment of pathologies arising from the aberrant immune responses (asthma, anaphylaxis, arthritis, etc.), Ganguly and Sainis sought to further characterize the immunogenic effects of the alkaloids in *T. indica*.<sup>153</sup> They found the alkaloid mixture mitigated delayed-type hypersensitivity (DTH) to sheep red blood cells (SRBC) and contact sensitivity to dinitrofluorobenzene (DNFB), indicating a suppression of T cell-mediated response. Additionally, the alkaloids inhibited mast cell degranulation and suppressed the discharge of pro-inflammatory mediators.

Lending further credence to their therapeutic value, prophylactic and remedial benefits were noted in the sensitized rodent models. Contrary to previous findings,<sup>151</sup> the alkaloids did not suppress primary humoral (IgM) immune response to SRBC.

The effect of the extracts was also investigated in rodent splenocytes.<sup>139</sup> Concanavalin A, a mitogen, was used to induce splenocyte proliferation as a model for the cellular immune response. Interestingly, a biphasic response was observed where proliferation was inhibited at high concentrations (>300 pg/mL) and stimulated at lower concentrations (19-300 pg/mL). Both T cells and macrophages were shown to be perturbed by the alkaloid extracts. This result is reminiscent of cryptopleurine's dual effect on motor nerve sprouting reported by Hoffman in 1952.<sup>154</sup> In addition to T cell mitogenic inhibition, IL-2 secretion was independently inhibited whereas IL-1 production increased. The alkaloids also activated macrophages, and hence were thought to exert their immunosuppressive activity via production of reactive oxygen and nitrogen species.

The molecular basis for *T. indica's* anti-inflammatory properties is not straightforward. These preclinical studies have provided insight into the pharmacological mechanisms by which *T. indica* alleviate asthma. The use of raw plant material or plant extracts, however, muddies the water in discussions of the mechanism of action. The chemical composition of any plant is a complex mixture of biomolecules, and although the most profound effects are often attributed to the most active component, it is conceivable that a few components, if not many, contribute to the overall physiological outcome. A second challenge comes from the variation among plants of the same species. It is well known that the subtle environmental factors can dramatically change the

biochemical landscape within a given plant species, thus, reproducibility may be difficult to attain.

### **2.1.5 Biology of the phenanthropiperidines**

Investigation of the pure alkaloidal constituents of *T. indica* has resolved some of the ambiguities that arise from studying complex botanical mixtures. Tylophorine, perhaps due to its high abundance (0.015-0.036%),<sup>103,131,155</sup> early discovery, and ease of synthesis, has received the most attention in this regard. The physiological effects of tylophorine are not subtle. The earliest isolation attempts report severe contact dermatitis and blistering upon exposure to the alkaline extracts of *T. indica*.<sup>103</sup> Notably, other plant species containing phenanthropiperidines and the alkaloids themselves are notorious for their vesicant properties.<sup>117,121</sup> These harmful effects have not deterred scientists from exploring their therapeutic potential for treating cancer, arthritis, and skin lesions (ironically). With the already established anti-inflammatory, immunosuppressive and cytotoxic properties of the plant extracts, it was fitting that the pure alkaloids should be appropriated in the same therapeutic areas. The following section will discuss these effects and their underlying causes.

#### **2.1.5.1 Anticancer activity**

##### **2.1.5.1.1 *In vitro* studies**

In the decades that followed tylocrebrine's failure in the clinic, medicinal interest in tylocrebrine and other phenanthropiperidines declined. Recently, however, there has been a resurgence of interest in these natural products and their analogs. This can be traced back to a NCI 60 tumor cell-line screen in the early 1990's that included several phenanthropiperidine alkaloids. To date, eleven naturally occurring phenanthropiperidines have been examined by the NCI.<sup>156</sup> Contrary to earlier speculation, many of these alkaloids have been found to possess potent and broad-spectrum cytotoxicity. Several members of this class were found to inhibit tumor cell growth with GI<sub>50</sub> values in the low nanomolar to subnanomolar range across the 60-cell line panel, on par with currently used anticancer drugs.<sup>156</sup>

Since the disclosure of this finding, the phenanthropiperidines have been actively pursued as leads for anticancer drugs. Thus far, a vast majority of these studies have been limited to *in vitro* antiproliferative experiments, and the NCI has acquired a majority of this data. A cross section of the 60 tumor cell line panel screen results is shown in Table 2-3. From this it should be clear the alkaloids are endowed with activity and have little discrimination between cell lines. This is demonstrated by the fact that the GI<sub>50</sub> ranges over the 60-cell line panel show moderate selectivity between the most and least susceptible cell lines. For example, there is only a 40-fold difference between tylophorine's growth inhibition in the most and least susceptible cell lines. To give this some perspective, paclitaxel, under the same assay, has a GI<sub>50</sub> range of 2 to 1000 nM, a 500-fold difference

**Table 2-3.** Growth inhibitory activity for NCI tested phenanthropiperidines

| Compound            | NSC #  | 60-cell line panel GI <sub>50</sub> (nM) |           | individual cell line GI <sub>50</sub> (nM) |                                      |                   |                  |
|---------------------|--------|--|-----------|--|--------------------------------------|-------------------|------------------|
|                     |        | Mean                                     | range     | CEM  | A549                                 | MCF-7             | HCT 116          |
| tylophorine         | 71735  | 17.5                                     | 10-400    | 10, 15 <sup>a</sup>                        | 10                                   | 10                | 10               |
| antofine            | NA     | ND                                       | ND        | 5.2 <sup>a</sup>                           | 10.4, <sup>b</sup> 0.44 <sup>c</sup> | 12.4 <sup>c</sup> | 9.9 <sup>b</sup> |
| cryptopleurine      | 19912  | 5.1                                      | 1.3-50    | 5.0, 2 <sup>a</sup>                        | 5.0                                  | 5.0               | 5.0              |
| tylocrebrine        | 60387  | 29.5                                     | 10-126    | 25   | 25                                   | 50                | 25               |
| DCB-3503            | 716802 | 29.1                                     | 10-160    | 25   | 25                                   | 16                | 20               |
| tylophorine         | 100055 | 57.6                                     | 10-500    | 40   | 63                                   | 63                | 40               |
| demethyltylophorine | 94739  | 1.13                                     | 1.0-16    | 1.0  | 1.0                                  | 1.0               | 1.0              |
| tyloindicine F      | 650393 | 0.10                                     | 0.1-0.1   | 0.10                                       | 0.10                                 | 0.10              | 0.10             |
| tyloindicine G      | 650394 | 0.10                                     | 0.10-0.16 | 0.10                                       | 0.10                                 | 0.10              | 0.10             |
| tyloindicine H      | 650395 | 0.50                                     | 0.10-20   | 0.40                                       | 0.63                                 | 3.98              | 0.50             |
| tyloindicine I      | 650396 | 4.40                                     | 1.6-20    | 3.16                                       | 3.98                                 | 5.01              | 2.0              |

CEM = leukemia, A549 = non-small cell lung carcinoma, MCF-7 = breast carcinoma, HCT 116 = colon carcinoma, NA = not applicable, ND = No data. Unless otherwise specified, data were gathered from NCI-DTP database. <sup>a</sup>Gao *et al.*<sup>152</sup> <sup>b</sup>Fu *et al.*<sup>153</sup> <sup>c</sup>Su *et al.*<sup>154</sup>

A second observation that can be made from *in vitro* studies is that these alkaloids retain their potency in drug-sensitive and multidrug-resistant (MDR) cell lines (Table 2-4).<sup>125,157-159</sup> Antofine and several closely related analogs have equipotent growth inhibitory activity against common and MDR cell lines.<sup>157,159</sup> Moreover, in a KB cell line panel containing strains resistant to many of the conventional anticancer drugs, tylophorine and its hydroxylated analog (DCB-3503) showed no bias in its antiproliferative effects.<sup>158</sup> Besides the obvious pertinence to therapeutic utility, this suggests that these alkaloids have a different mechanism of action from drugs that are ineffective in these refractory cell lines.

**Table 2-4.** Growth inhibitory activity in drug-resistant cell lines (Gao *et al.*)<sup>158</sup>

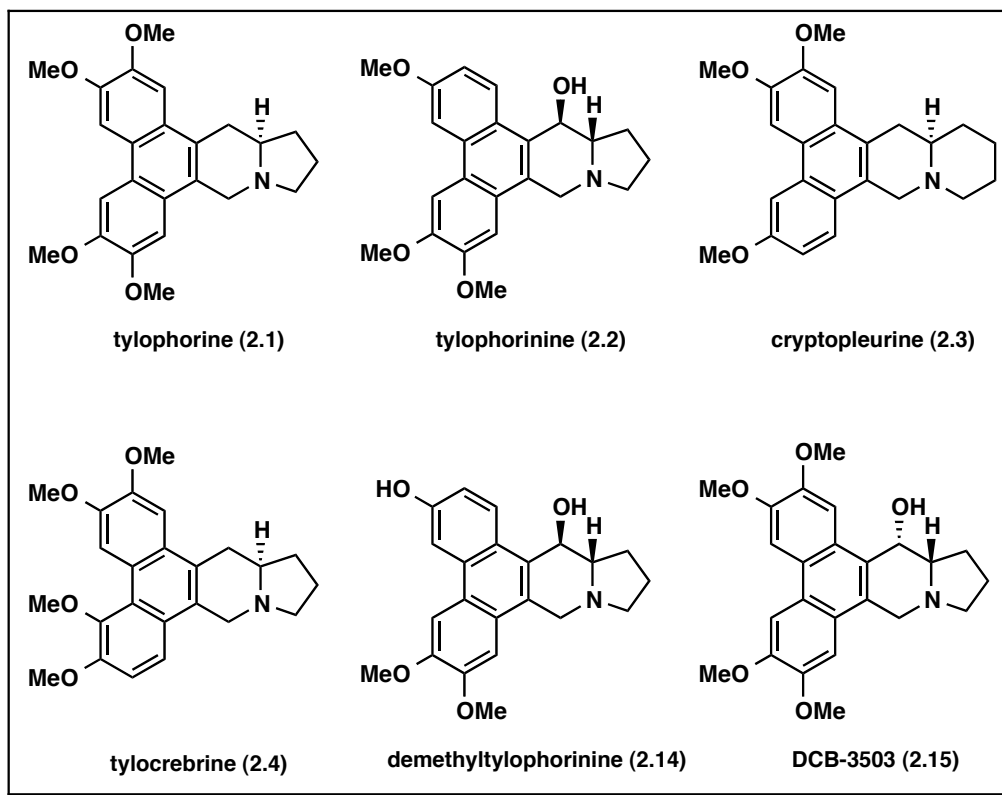
| cell line     | biochemical changes | resistance                            | tylophorine          | GI <sub>50</sub> (nM) |                                   |
|---------------|---------------------|---------------------------------------|----------------------|-----------------------|-----------------------------------|
|               |                     |                                       |                      | DCB-3503              | antofine                          |
| KB (parental) | –                   | –                                     | 12, 214 <sup>a</sup> | 28                    | 16, <sup>a</sup> 1.2 <sup>b</sup> |
| KB-MDR        | gp 170 ↑            | VP-16, taxol, adriamycin, vincristine | 14                   | 26                    | –                                 |
| KB-7D         | Topo II ↓, MRP ↑    | VP-16, adriamycin, vincristine        | 12                   | 45                    | –                                 |
| KB-7D-Rev     | Topo II ↓           | VP-16, adriamycin                     | 11                   | 25                    | –                                 |
| KB-Ha-R       | RR ↑, dCK ↓         | HU, araC, gemcitabine                 | 25                   | 36                    | –                                 |
| KB-Ha-Rev     | dCK ↓               | araC, gemcitabine                     | 16                   | 28                    | –                                 |
| KB-100        | Topo I ↓, XRCC1 ↑   | CPT, topotecan, SN-38                 | 20                   | 38                    | –                                 |
| KB-100-Rev    | Topo I ↓            | CPT, topotecan, SN-38                 | 10                   | 41                    | –                                 |
| KB-VI         | P-gp ↑              | adriamycin, vinblastine, colchicine   | 173                  | –                     | 14, <sup>a</sup> 2.3 <sup>b</sup> |

<sup>a</sup>Staerk *et al.*<sup>155</sup> <sup>b</sup>Su *et al.*<sup>154</sup> MRP = multidrug resistant protein, VP-16, etoposide; HU = hydroxyurea, RR = ribonucleotide reductase, dCK = deoxycytidine kinase, Topo II = topoisomerase II, Topo I = topoisomerase I, XRCC1 = X-ray repair cross-contemplating gene I protein, araC = aravinoside cytosine, CPT = camptothecin, SN-38 = 7-ethyl-10-hydroxycamptothecin, P-gp = P-glycoprotein.

#### 2.1.5.1.2 *In vivo* studies

Much of our current understanding for the phenanthropiperidine's effects *in vivo* arises from early investigations. As seen already, tylophorine, cryptopleurine, and tylocrebrine were the first members to be discovered. Out of these three alkaloids, cryptopleurine was found to be the most toxic and tylophorine the least. The general toxicity of cryptopleurine was studied in guinea pig, rat, mouse, rabbit, cat, and dog.<sup>117</sup> The alkaloid proved to be lethal at doses as low as 1 mg/kg with little regard for species or mode of administration.





**Figure 2-5.** Structures of NCI tested phenanthropiperidines.

In spite of its potent toxicity, low doses of cryptopleurine (1 mg/kg *i.p.* twice daily for 8 days) inhibited the growth of Ehrlich ascite tumors in mice without killing the host.<sup>160</sup> Preclinical studies at the NCI showed cryptopleurine only had marginal activity *in vivo* (Table 2-5).<sup>104</sup> On the other hand, tylocrebrine was shown to be a more promising lead and became a clinical candidate soon after its discovery. Still, this alkaloid was found to be inactive in sarcoma 180, adenocarcinoma 755, B16 melanoma, Lewis lung, P1534 leukemia, and Walker 256 carcinoma models. It was tylocrebrine's structural novelty and superior efficacy to tylophorine and cryptopleurine<sup>161</sup> in lymphoid leukemia

L1210 mouse models that justified its entry into clinical trials.<sup>104</sup> In 1966, less than a year after the clinical trials began, the studies were discontinued before its full therapeutic value could be determined. Suffness reported that this determination was due to central nervous system (CNS) toxicity.<sup>104</sup> The toxicity became evident when the patient experienced ataxia and disorientation. Unfortunately, the original clinical data from these studies could not be obtained.<sup>104</sup>

**Table 2-5.** Best NCI *in vivo* results for L1210 leukemia model

| <b>compound</b>              | <b>NSC #</b>  | <b>dose (mg/kg)</b> | <b>life extension (%)</b> |
|------------------------------|---------------|---------------------|---------------------------|
| <b>(R)-cryptopleurine</b>    | <b>19912</b>  | <b>1</b>            | <b>140</b>                |
| <b>(R)-tylocrebrine</b>      | <b>60387</b>  | <b>20</b>           | <b>155</b>                |
| <b>(S)-tylophorine</b>       | <b>717335</b> | <b>30</b>           | <b>150</b>                |
| <b>tylophorinine</b>         | <b>100055</b> | <b>6</b>            | <b>130</b>                |
| <b>demethyltylophorinine</b> | <b>94739</b>  | <b>15</b>           | <b>153</b>                |

Recently, a synthetic analog of tylophorine DCB-3503 was reported to have promising anticancer properties *in vivo* and a novel mechanism of action. Interestingly, despite being less active than tylophorine *in vitro*, DCB-3503 was far superior when tested *in vivo* in the hollow-fiber assay.<sup>158</sup> DCB-3503 also caused significant tumor growth suppression in nude mice with HepG2 xenographs, increasing the tumor doubling time from 2-3 days and 5-6 days.<sup>158</sup> These findings have given rise to extensive pharmacological studies that further supports development of this lead for clinical use.<sup>162-</sup>

### 2.1.5.1.3 Structure-activity relationships (SAR)

The size of the phenanthropiperidine class has grown significantly since the isolation of tylophorine in 1935. There are over 100 members in this class, including seco-analogs and other closely related isomers from which SARs have emerged. For the purpose of this discussion, approximately one hundred and fifty natural and synthetic analogs will be considered. The compounds are listed in Table 2-6 and are ordered by scaffold (Figure 2-6). The analogs mean GI<sub>50</sub>'s are noted in up to twelve cell lines or, for those tested in the NCI's 60-tumor cell line panel, the mean GI<sub>50</sub> value in the 60-tumor cell line is shown.<sup>125,156,164-188</sup> It should be noted that these compounds may not have been tested in the same assay or even in the same cell lines. Moreover since internal standards were rarely used, normalization of the data is impossible.

**Table 2-6.** Structure and average GI<sub>50</sub> of phenanthropiperidines against cancer cells

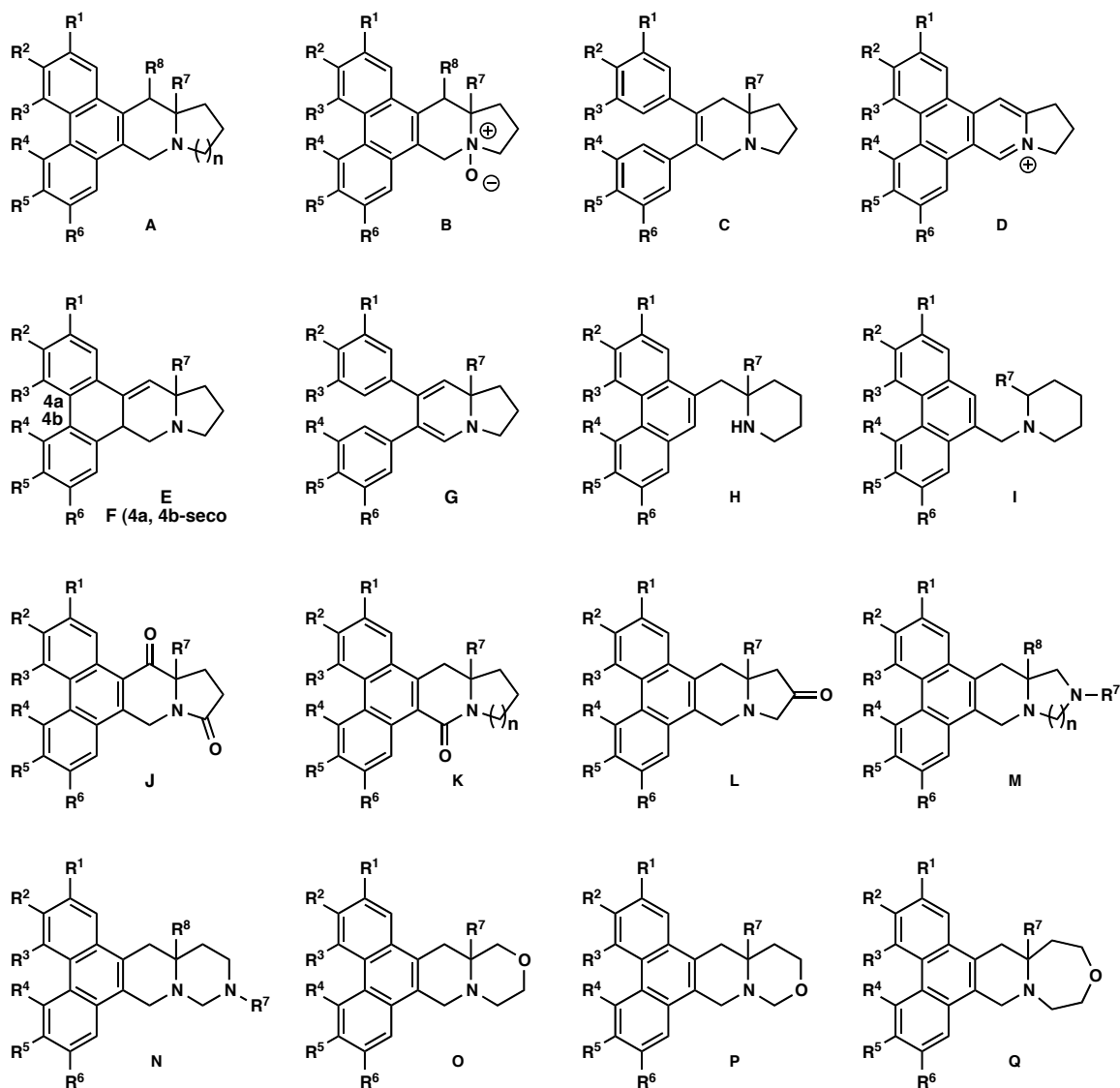
| entry | common name                | scaffold <sup>a</sup> | R <sup>1</sup>       | R <sup>2</sup> | R <sup>3</sup> | R <sup>4</sup> | R <sup>5</sup> | R <sup>6</sup> | R <sup>7</sup> | R <sup>8</sup> | n | GI <sub>50</sub> (nM) |                 |
|-------|----------------------------|-----------------------|----------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|---|-----------------------|-----------------|
|       |                            |                       |                      |                |                |                |                |                |                |                |   | mean <sup>b</sup>     | SD <sup>c</sup> |
| 1     | -                          | A                     | H                    | OH             | OMe            | H              | OMe            | OMe            | S-H            | S-OH           | 1 | < 0.2                 | -               |
| 2     | -                          | A                     | H                    | OH             | OMe            | H              | OMe            | OMe            | S-H            | H              | 1 | < 0.5                 | -               |
| 3     | -                          | A                     | OMe                  | OMe            | H              | OMe            | OH             | H              | S-H            | H              | 1 | < 0.5                 | -               |
| 4     | (±)-6-O-demethylantofine   | A                     | OMe                  | OMe            | H              | H              | OH             | H              | rac            | H              | 1 | 1.2                   | 0.2             |
| 5     | (±)-cryptopleurine         | A                     | OMe                  | OMe            | H              | H              | OMe            | H              | rac            | H              | 2 | 1.3                   | 0.8             |
| 6     | demethyltylophorinine      | A                     | H                    | OH             | H              | H              | OMe            | OMe            | S-H            | R-OH           | 1 | 1.4                   | 2.2             |
| 7     | -                          | A                     | H                    | OH             | OMe            | H              | OH             | OMe            | S-H            | H              | 1 | 1.7                   | 1.0             |
| 8     | (R)-7-desmethyltylophorine | A                     | OMe                  | OMe            | H              | H              | OMe            | OH             | R-H            | H              | 1 | 5.5                   | 7.2             |
| 9     | (R)-cryptopleurine         | A                     | OMe                  | OMe            | H              | H              | OMe            | H              | R-H            | H              | 2 | 6.0                   | 6.8             |
| 10    | (R)-6-O-demethylantofine   | A                     | OMe                  | OMe            | H              | H              | OH             | H              | R-H            | H              | 1 | 8.5                   | -               |
| 11    | tylophoridicine E          | A                     | H                    | OH             | H              | H              | OMe            | OMe            | S-H            | S-OH           | 1 | < 10                  | -               |
| 12    | (±)-antofine               | A                     | OMe                  | OMe            | H              | H              | OMe            | H              | rac            | H              | 1 | 13                    | 14              |
| 13    | (R)-antofine               | A                     | OMe                  | OMe            | H              | H              | OMe            | H              | R-H            | H              | 1 | 13                    | 8.5             |
| 14    | deoxypergularine           | A                     | H                    | OMe            | H              | H              | OMe            | OMe            | rac            | H              | 1 | 23                    | 14              |
| 15    | -                          | A                     | OMe                  | OMe            | H              | H              | i-PrO          | H              | rac            | H              | 1 | 28                    | 11              |
| 16    | boehmeriasin A             | A                     | H                    | OMe            | H              | H              | OMe            | OMe            | rac            | H              | 2 | 30                    | 80              |
| 17    | -                          | A                     | OMe                  | OH             | H              | H              | OMe            | H              | rac            | H              | 1 | 30                    | 17              |
| 18    | DCB-3503                   | A                     | OMe                  | OMe            | H              | H              | OMe            | OMe            | S-H            | S-OH           | 1 | 32                    | 17              |
| 19    | isotylocrebrine            | A                     | H                    | OMe            | OMe            | H              | OMe            | OMe            | S-H            | H              | 1 | 33                    | 17              |
| 20    | (R)-tylocrebrine           | A                     | OMe                  | OMe            | H              | OMe            | OMe            | H              | R-H            | H              | 1 | 35                    | 24              |
| 21    | -                          | A                     | OMe                  | i-PrO          | H              | H              | OMe            | H              | rac            | H              | 1 | 39                    | 15              |
| 22    | (S)-tylophorine            | A                     | OMe                  | OMe            | H              | H              | OMe            | OMe            | S-H            | H              | 1 | 61                    | 170             |
| 23    | demethoxyantofine          | A                     | OMe                  | OMe            | H              | H              | H              | H              | rac            | H              | 1 | > 70                  | -               |
| 24    | -                          | A                     | OMe                  | OMe            | H              | H              | OMe            | H              | R-H            | H              | 3 | 79                    | 78              |
| 25    | -                          | A                     | OH                   | OMe            | H              | H              | OMe            | H              | rac            | H              | 1 | > 80                  | -               |
| 26    | DCB-3501                   | A                     | OMe                  | OMe            | H              | H              | OMe            | OMe            | S-H            | R-OH           | 1 | 110                   | 52              |
| 27    | 7-methoxycryptopleurine    | A                     | OMe                  | OMe            | H              | H              | OMe            | OMe            | rac            | H              | 2 | 118                   | 11              |
| 28    | (R)-tylophorine            | A                     | OMe                  | OMe            | H              | H              | OMe            | OMe            | R-H            | H              | 1 | 150                   | 120             |
| 29    | tylophorinine              | A                     | H                    | OMe            | H              | H              | OMe            | OMe            | S-H            | R-OH           | 1 | 270                   | 1100            |
| 30    | (R)-ficuseptine C          | A                     | -OCH <sub>2</sub> O- | H              | H              | OMe            | H              | R-H            | H              | H              | 1 | 280                   | 110             |
| 31    | (±)-ficuseptine C          | A                     | -OCH <sub>2</sub> O- | H              | H              | OMe            | H              | rac            | H              | H              | 1 | 520                   | 200             |
| 32    | DCB-3506                   | A                     | OMe                  | OH             | H              | H              | OMe            | OMe            | S-H            | S-OH           | 1 | > 600                 | -               |
| 33    | (±)-tylophorine            | A                     | OMe                  | OMe            | H              | H              | OMe            | OMe            | rac            | H              | 1 | 620                   | 670             |
| 34    | -                          | A                     | i-PrO                | OMe            | H              | H              | OMe            | H              | rac            | H              | 1 | > 900                 | -               |
| 35    | tylophorinidine            | A                     | H                    | OMe            | H              | H              | OH             | OMe            | S-H            | S-OH           | 1 | 2000                  | 3000            |
| 36    | -                          | A                     | H                    | H              | H              | H              | H              | H              | rac            | H              | 1 | 4000                  | 2100            |
| 37    | deoxytylophorinidine       | A                     | H                    | OMe            | H              | H              | OH             | OMe            | R-H            | H              | 1 | 4600                  | 9100            |
| 38    | (S)-deoxytylophorinidine   | A                     | H                    | OMe            | H              | H              | OMe            | OMe            | S-H            | H              | 1 | 383                   | 76              |
| 39    | -                          | A                     | OMe                  | OMe            | H              | H              | H              | OMe            | S-H            | H              | 1 | 234                   | 78              |
| 40    | -                          | A                     | OH                   | H              | H              | H              | OMe            | OMe            | S-H            | S-OH           | 1 | 18                    | 22              |
| 41    | -                          | A                     | H                    | H              | OH             | H              | OMe            | OMe            | S-H            | S-OH           | 1 | 29                    | 46              |
| 42    | -                          | A                     | H                    | H              | H              | H              | OMe            | OMe            | S-H            | S-OH           | 1 | 12                    | 5.2             |
| 43    | -                          | A                     | H                    | H              | H              | H              | OMe            | OMe            | R-H            | R-OH           | 1 | 1070                  | 830             |
| 44    | -                          | A                     | H                    | Et             | H              | H              | OMe            | OMe            | S-H            | S-OH           | 1 | 291                   | 233             |
| 45    | -                          | A                     | H                    | Et             | H              | H              | OMe            | OMe            | R-H            | R-OH           | 1 | 726                   | 749             |
| 46    | -                          | A                     | H                    | F              | H              | H              | OMe            | OMe            | S-H            | S-OH           | 1 | 27                    | 20              |
| 47    | -                          | A                     | H                    | F              | H              | H              | OMe            | OMe            | R-H            | R-OH           | 1 | 284                   | 320             |
| 48    | -                          | A                     | H                    | OAc            | H              | H              | OMe            | OMe            | S-H            | S-OH           | 1 | 1.1                   | 0.81            |
| 49    | -                          | A                     | H                    | OH             | H              | H              | H              | H              | S-H            | S-OH           | 1 | 926                   | 902             |
| 50    | -                          | A                     | H                    | OH             | H              | H              |                |                | S-H            | S-OH           | 1 | 72                    | 57              |

| entry | common name                  | scaffold <sup>a</sup> | R <sup>1</sup> | R <sup>2</sup> | R <sup>3</sup> | R <sup>4</sup> | R <sup>5</sup>                        | R <sup>6</sup> | R <sup>7</sup> | R <sup>8</sup>    | n   | GI <sub>50</sub> (nM) |                 |
|-------|------------------------------|-----------------------|----------------|----------------|----------------|----------------|---------------------------------------|----------------|----------------|-------------------|-----|-----------------------|-----------------|
|       |                              |                       |                |                |                |                |                                       |                |                |                   |     | mean <sup>b</sup>     | SD <sup>c</sup> |
| 51    | -                            | A                     | H              | OH             | H              | H              | -OC(CH <sub>3</sub> ) <sub>2</sub> O- | S-H            | S-OH           | 1                 | 719 | 221                   |                 |
| 52    | -                            | A                     | H              | OH             | H              | H              | OEt                                   | OEt            | S-H            | S-OH              | 1   | 4.3                   | 5.8             |
| 53    | -                            | A                     | OH             | H              | H              | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 26                    | 5.6             |
| 54    | -                            | A                     | H              | H              | OH             | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 154                   | 258             |
| 55    | -                            | A                     | H              | OAc            | H              | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 2.7                   | 0.8             |
| 56    | -                            | A                     | H              | H              | H              | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 52                    | 4.5             |
| 57    | -                            | A                     | H              | F              | H              | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 53                    | 5.6             |
| 58    | -                            | A                     | H              | Et             | H              | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 440                   | 41              |
| 59    | -                            | A                     | H              | OH             | H              | H              | OEt                                   | OEt            | S-H            | H                 | 1   | 54                    | 23              |
| 60    | -                            | A                     | H              | OH             | H              | H              | -OCH <sub>2</sub> O-                  | S-H            | H              | 1                 | 549 | 286                   |                 |
| 61    | -                            | A                     | H              | OH             | H              | OMe            | OMe                                   | OMe            | S-H            | S-OH              | 1   | 2700                  | 2000            |
| 62    | -                            | A                     | H              | OH             | H              | OMe            | OMe                                   | OMe            | R-H            | R-OH              | 1   | 9.9                   | 3.4             |
| 63    | -                            | A                     | H              | OH             | H              | H              | F                                     | H              | S-H            | S-OH              | 1   | 900                   | 600             |
| 64    | -                            | A                     | H              | OH             | H              | H              | OMe                                   | OMe            | S-H            | S-OAc             | 1   | 6.3                   | 5.6             |
| 65    | -                            | A                     | H              | OH             | H              | H              | OMe                                   | OMe            | rac-Me         | rac-OH            | 1   | 4100                  | 3500            |
| 66    | -                            | A                     | OMe            | OMe            | H              | H              | OH                                    | OMe            | S-H            | S-OH              | 1   | 87                    | 72              |
| 67    | -                            | A                     | Cl             | OMs            | H              | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 16                    | 1.6             |
| 68    | -                            | A                     | H              | OMs            | Cl             | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 3.9                   | 4.3             |
| 69    | -                            | A                     | F              | OMs            | H              | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 9.5                   | 5.4             |
| 70    | -                            | A                     | Me             | OMs            | Me             | H              | OMe                                   | OMe            | S-H            | H                 | 1   | 134                   | 77              |
| 71    | -                            | A                     | H              | OH             | H              | H              | OMe                                   | OMe            | S-H            | rac-NH            | 1   | 2600                  | 3600            |
| 72    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | S-NH <i>n</i> -Pr | 1   | 6600                  | -               |
| 73    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | R-NH <i>n</i> -Pr | 1   | 5400                  | -               |
| 74    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | S-NH <i>i</i> -Pr | 1   | 5200                  | -               |
| 75    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | R-NH <i>i</i> -Pr | 1   | 5200                  | -               |
| 76    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | S-NHc-Pentyl      | 1   | 4800                  | -               |
| 80    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | S-NHBn            | 1   | 5600                  | -               |
| 81    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | R-NHBn            | 1   | 4400                  | -               |
| 82    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | S-NH              | 1   | 716                   | 1450            |
| 83    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | R-NH              | 1   | 1100                  | 2300            |
| 84    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | S-OMe             | 1   | 276                   | 444             |
| 85    | -                            | A                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | R-OMe             | 1   | 5200                  | -               |
| 86    | -                            | B                     | H              | OH             | OMe            | H              | OMe                                   | OMe            | S-H            | S-OH              | -   | 13.0                  | 6.2             |
| 87    | tylophorine- <i>N</i> -oxide | B                     | OMe            | OMe            | H              | H              | OMe                                   | OMe            | R-H            | H                 | -   | 91.1                  | 62.7            |
| 88    | -                            | B                     | OMe            | OMe            | H              | H              | OMe                                   | H              | R-H            | R-OH              | -   | 130                   | -               |
| 89    | antofine- <i>N</i> -oxide    | B                     | OMe            | OMe            | H              | H              | OMe                                   | H              | R-H            | H                 | -   | 140                   | -               |
| 90    | -                            | B                     | OMe            | OMe            | H              | H              | OMe                                   | OH             | R-H            | H                 | -   | 550                   | 320             |
| 91    | tylophoridine C              | B                     | H              | OMe            | H              | H              | OH                                    | OMe            | S-H            | S-OH              | -   | > 9200                | -               |
| 92    | tylophoridine F              | B                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | R-OH              | -   | > 11000               | -               |
| 93    | ( <i>R</i> )-secoantofine    | C                     | OMe            | OMe            | H              | H              | OMe                                   | H              | R-H            | -                 | -   | 2500                  | -               |
| 94    | -                            | C                     | H              | OMe            | H              | H              | OH                                    | OMe            | S-H            | -                 | -   | 2800                  | 2700            |
| 95    | -                            | C                     | OMe            | OMe            | H              | H              | OH                                    | OMe            | S-H            | -                 | -   | 8900                  | 2200            |
| 96    | -                            | C                     | OMe            | OMe            | H              | H              | OMe                                   | OMe            | S-H            | -                 | -   | 18800                 | 3450            |
| 97    | -                            | C                     | H              | OMe            | H              | H              | OMe                                   | OMe            | S-H            | -                 | -   | 2300                  | 830             |
| 98    | dehydroantofine              | D                     | OMe            | OMe            | H              | H              | OMe                                   | H              | -              | -                 | -   | 930                   | 580             |
| 99    | dehydrotylophorine           | D                     | OMe            | OMe            | H              | H              | OMe                                   | OMe            | -              | -                 | -   | > 50000               | -               |
| 100   | tyloindicine G               | E                     | OMe            | OMe            | H              | H              | OMe                                   | OMe            | OH             | -                 | -   | 0.1                   | -               |

| entry | common name    | scaffold <sup>a</sup> | R <sup>1</sup> | R <sup>2</sup> | R <sup>3</sup> | R <sup>4</sup> | R <sup>5</sup> | R <sup>6</sup> | R <sup>7</sup>                     | R <sup>8</sup> | n | GI <sub>50</sub> (nM) |                 |
|-------|----------------|-----------------------|----------------|----------------|----------------|----------------|----------------|----------------|------------------------------------|----------------|---|-----------------------|-----------------|
|       |                |                       |                |                |                |                |                |                |                                    |                |   | mean <sup>b</sup>     | SD <sup>c</sup> |
| 101   | tyloindicine F | F                     | H              | OMe            | H              | H              | OMe            | OMe            | OH <sup>d</sup>                    | -              | - | 0.1                   | -               |
| 102   | tyloindicine I | G                     | OMe            | OMe            | OH             | H              | OMe            | OMe            | H                                  | -              | - | 4.9                   | 2.9             |
| 103   | -              | H                     | OMe            | OMe            | H              | H              | OMe            | H              | R-H                                | -              | - | 1100                  | 600             |
| 104   | -              | I                     | OMe            | OMe            | H              | H              | OMe            | H              | S-Me                               | -              | - | > 10000               | -               |
| 105   | -              | I                     | OMe            | OMe            | H              | H              | OMe            | H              | R-Me                               | -              | - | > 11000               | -               |
| 106   | -              | J                     | OMe            | OMe            | H              | H              | OMe            | OMe            | rac                                | -              | - | 22000                 | 18000           |
| 107   | -              | K                     | OMe            | OMe            | H              | H              | OMe            | OMe            | rac                                | -              | 1 | >50000                | -               |
| 108   | -              | K                     | OMe            | OMe            | H              | H              | OMe            | OMe            | rac                                | -              | 2 | 32000                 | 10000           |
| 109   | -              | L                     | OMe            | OMe            | H              | H              | OMe            | H              | R-H                                | -              | - | 480                   | 180             |
| 110   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | Me                                 | S-H            | 1 | 1400                  | 630             |
| 111   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | CO <sub>2</sub> Me                 | S-H            | 2 | 11600                 | 600             |
| 112   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | Ac                                 | S-H            | 2 | 13200                 | 1800            |
| 113   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | Ms                                 | S-H            | 2 | 16700                 | 1500            |
| 114   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | CH <sub>2</sub> CO <sub>2</sub> Me | S-H            | 2 | 14500                 | -               |
| 115   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | CH <sub>2</sub> c-Pr               | S-H            | 2 | 2600                  | 2900            |
| 116   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | c-Pr                               | S-H            | 2 | 15700                 | -               |
| 117   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | Bz                                 | S-H            | 2 | 16300                 | -               |
| 118   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | 2'-OH-Et                           | S-H            | 2 | 10700                 | 3200            |
| 119   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | PO(OMe)                            | S-H            | 2 | 11300                 | 2800            |
| 120   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | Me                                 | S-H            | 2 | 7200                  | 1900            |
| 121   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | Bn                                 | S-H            | 2 | 16000                 | -               |
| 122   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | CONMe                              | R-H            | 2 | 12700                 | 1100            |
| 123   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | i-Bu                               | R-H            | 2 | 11600                 | 1300            |
| 124   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | Bz                                 | R-H            | 2 | 12000                 | 3600            |
| 125   | -              | M                     | OMe            | OMe            | H              | H              | OMe            | H              | CH <sub>2</sub> c-Pr               | R-H            | 2 | 13400                 | -               |
| 126   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | 2'-OH-Et                           | S-H            | - | 5000                  | 4200            |
| 127   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | Ph                                 | S-H            | - | > 20000               | -               |
| 128   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | i-Bu                               | S-H            | - | 2600                  | 1700            |
| 129   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | Bn                                 | S-H            | - | >20000                | -               |
| 130   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | c-Pr                               | S-H            | - | 9000                  | 4700            |
| 131   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | NMe                                | S-H            | - | 2600                  | 600             |
| 132   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | i-Bu                               | R-H            | - | 3900                  | 2800            |
| 133   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | NMe                                | R-H            | - | 4000                  | 1300            |
| 134   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | 2'-OH-Et                           | R-H            | - | 2100                  | -               |
| 135   | -              | N                     | OMe            | OMe            | H              | H              | OMe            | H              | CH <sub>2</sub> c-Pr               | R-H            | - | 2500                  | 2100            |
| 136   | -              | O                     | OMe            | OMe            | H              | H              | OMe            | H              | S-H                                | -              | - | 34                    | 25              |
| 137   | -              | O                     | OMe            | OMe            | H              | H              | OMe            | H              | R-H                                | -              | - | 7800                  | 4400            |
| 138   | -              | P                     | OMe            | OMe            | H              | H              | OMe            | H              | S-H                                | -              | - | 1700                  | 170             |
| 139   | -              | P                     | OMe            | OMe            | H              | H              | OMe            | H              | R-H                                | -              | - | 1300                  | 110             |
| 140   | -              | Q                     | OMe            | OMe            | H              | H              | OMe            | H              | R-H                                | -              | - | 79                    | 78              |
| 141   | -              | Q                     | OMe            | OMe            | H              | H              | OMe            | H              | S-H                                | -              | - | 62                    | 43              |

<sup>a</sup> See Fig. 2-6. <sup>b</sup> Average GI<sub>50</sub> in HepG2, Panc-1, CEM, A549, MCF-7, KB, KB-VI, KB-3-1, HCT 116, HT-1080, MDA-MB-231, COLO-205, NUGC-3 and HONE-1. <sup>c</sup> Standard deviation is calculated from the set of GI<sub>50</sub> values reported for each compound in the indicated cell lines without consideration for the standard deviations determined upon acquisition of the original data. Standard deviation is only included for compounds which have been tested in three or more cell lines did not have any GI<sub>50</sub> values reported as inequalities (*i.e.* GI<sub>50</sub> > 50,000 nM). <sup>d</sup> Unknown stereochemistry

Another concern is that even among the same cell lines there is a significant amount of variation between assays. For example, in A549 cells, antofine (entry 13) was reported to have a  $GI_{50}$  of 25 nM in one report and 0.44 nM in another. This 50-fold discrepancy demonstrates the variation that can exist between independently acquired data in anti-proliferation assays. To this end, standard deviations have been included, where possible, to show where the possible variations exist.



**Figure 2-6.** Scaffolds of phenanthropiperidines and analogs.

When evaluating the data in Table 2-6, it becomes obvious that compounds with the phenanthropiperidine scaffold (A) are the most consistently active (entries 1-85). Also, variations among the phenanthrene system (rings A-C) are the most extensively studied. Seco-analogs of the phenanthrene system tend to be much less active as shown by seco-antofine (entry 93). However, in the case of tyloindicines F and I this is not the case. Even though the A and C rings are disconnected they are extremely cytotoxic (entries 101 and 102). DCB-3502 (dearomatized version of entry 22) shows that disrupting the aromaticity in the B ring can also reduce activity.<sup>173,189</sup> The location and type of substituents on the phenanthrene system can have a profound effect on the activity.<sup>157,172,190</sup> Formulating a strict phenanthrene SAR without considering the rest of the molecule leads to conflicting data. This fact is shown when all the methoxy or hydroxy substituents are removed; the naked scaffold is significantly less active (entry 36). From this data, it can be inferred that the presence of these substituents are necessary for activity.<sup>189</sup> Although this may be due to the presence of a H-bond donor or an electron rich arene, the incorporation of bioisosteres such as amines have not been investigated.

As stated earlier, the most extensively studied region of the phenanthropiperidines is the phenanthrene system. Suffice to say the phenanthrene system is sensitive toward modification and it is difficult to make generalizations. The 2,3,6-trisubstituted system seems to be favorable as shown by the significant cytotoxicity of antofine and cryptopleurine (entries 5, 9, 12 and 13). The exceptional cytotoxicity of 6-*O*-desmethylantofine<sup>167</sup> (entry 4) and (*R*)-7-desmethyltylophorine (entry 8) suggest that a hydroxy group at C6 ( $R^5$ ) on the phenanthrene system can be tolerated. However, depending on the scaffold C3-hydroxy groups ( $R^2$ ) will either increase or decrease



activity (entry 17 vs. 32). A systematic study of antofine's phenanthrene system was reported by Fu *et al.*<sup>190</sup> and they found that the C2-position ( $R^1$ ) was particularly sensitive toward modification. Replacement of the methoxy with a methylenedioxy bridge, hydroxy or isopropoxy group resulted in loss of activity (entries 13 vs. 23, 31 or 34). While replacing the methoxy groups at the C3 ( $R^2$ ) and C6 ( $R^5$ ) with hydroxy or isopropoxy was tolerated quite well (entries 13 vs. 17 or 21).

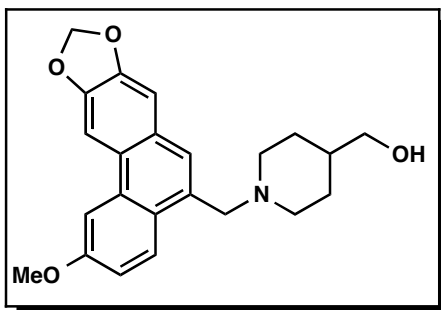
There is evidence that the stereochemistry at the bridgehead carbon contributes to activity. Surprisingly, only a few labs have tested both enantiomers simultaneously to allow for a legitimate comparison. Gao *et al.*<sup>173</sup> were able to synthesize both enantiomers of tylophorine and found that the (*S*)-isomer was 3-5 times more active than the (*R*)-isomer (entries 22 vs. 28) in HepG2, Panc-1, and CEM cells.<sup>173</sup> Separately (*R*)-antofine was tested with its racemate in A549, HCT 116, and HT-1080 cells. The (*R*)-enantiomer was found to be 3 times more active in all cell lines (entries 12 vs. 13).<sup>191</sup> From this data, it can be inferred that in the case of antofine the (*R*)-enantiomer is more active, contradictory to that of tylophorine. If this is the case, the phenanthrene substituents have differing effects on the activity of each enantiomer and the absolute stereochemistry is not uniform across the class.

Several modifications can be made on the D-ring without losing activity. A C14-hydroxy group ( $R^8$ ) seems to be well tolerated, with only a slight loss (entries 14 vs. 29) or a slight increase (entries 18 vs. 22) of activity. Even though some of the C14-hydroxylated analogs have slightly diminished activity, they are still some of the most potent members in the class (entries 1, 6, and 11). One direct comparison that can be made is that the (13a*S*,14*S*) diastereomer of the C14-hydroxylated tylophorine is more

active than the (13a*S*,14*R*) diastereomer (entries 18 vs. 26). However, demethyltylophorine features the same (13a*S*,14*R*) hydroxylated motif and has excellent cytotoxicity (entry 6). Acylation of the C14-hydroxyl also leads to a compound with potent cytotoxicity (entry 64). Replacement of the C14-hydroxyl with C14-amino groups generally led to a severe decrease in cytotoxicity (entries 71-83). Interestingly Gao *et al.* reported that DCB-3503 not only showed activity *in vitro*, but displayed superior activity *in vivo* when compared to its non-hydroxylated counterpart, tylophorine. This could prove to be beneficial in future endeavors.

Another question that needs to be addressed is whether a basic nitrogen is required for activity. Several N-oxide analogs have been synthesized and tested. Surprisingly, the *N*-oxides were only 2-10 fold less active, which suggests that a basic nitrogen may not be essential for activity (entries 86-92). There have also been some amide compounds synthesized, which were almost completely inactive (entries 107 and 108). Dehydro-analogs where the piperidine is aromatized to the pyridinium also resulted in a significant loss of activity (entries 98 and 99).

Scaffolds H and I (entries 103-105) have been designed to remove the D-ring completely. All permutations of this kind have reduced the anti-proliferative effects. However, the ease of synthesis of these analogs has enabled an extensive SAR study and resulted in only moderate cytotoxicity, such as PBT-1 (Figure 2-7). Even though compounds such as PBT-1 are structurally related, evidence suggests that these analogs operate under a distinct mechanism of action from typical phenanthropiperidines.<sup>173,181</sup>



**Figure 2-7.** Structure of PBT-1.

There is currently a lack of understanding of the SAR of the E-ring and whether it is required or can be modified like the D-ring. What is known is that the quinolizidine ring system is generally slightly more active than the indolizidine counterpart.<sup>180,189,192</sup> Yang *et al.* recently reported the synthesis and anti-proliferative activity of several E-ring analogs. A 7-membered analog of antofine was equipotent in A549 cells, but had decreased activity in DU145 and KB cells (entry 24). Incorporation of a ketone into antofine's E-ring resulted in a significant loss of activity (entry 109). An even greater loss of activity was observed when a nitrogen was incorporated into antofine or cryptopleurine's E-ring (entries 110-135).<sup>188</sup> However, introduction of an oxygen into the E-ring resulted in some active compounds (entries 136, 140, and 141).<sup>188</sup> Interestingly, the position of the oxygen and stereochemistry of the bridgehead carbon had a large effect on the activity of the E-ring analogs (entries 136-141).<sup>188</sup>

There is still a limited understanding of the SAR around the phenanthropiperidines. This is because most studies are confined to a uniform collection of analogs. Although the effect of substitution location has been explored, the type of substitution has not.

### **2.1.5.2 Mechanism of action**

The mechanism by which the phenanthropiperidines exert their anti-proliferative effects has been under investigation for some time. A COMPARE analysis was performed by the NCI, which suggested that their effects were through a unique mechanism and, potentially, a novel molecular target. From these studies, several targets have been proposed, but no one consenting mechanism. There is a possibility that the alkaloids operate under multiple mechanisms, because some of the possible targets identified are only disturbed at concentrations much higher than what is required for the desired biological effect. There is also some evidence that structural modifications may change the mechanism of action, making broad generalizations virtually impossible.

#### **2.1.5.2.1 Protein, DNA, and RNA biosynthesis inhibition**

By far the most extensively studied mechanism for the phenanthropiperidine's anti-cancer activity is protein biosynthesis inhibition. Starting in 1968, Donaldson *et al.* reported that tylophorine, tylocrebrine, and cryptopleurine inhibited protein synthesis in Ehrlich ascites-tumor cells, but did not markedly affect RNA synthesis.<sup>160</sup> According to their results cryptopleurine was the most active ( $IC_{50} = 20$  nM) and tylophorine was the least active ( $IC_{50} = 1000$  nM). Cryptopleurine's activity in eukaryotic systems failed to translate to prokaryotic systems as cryptopleurine did not inhibit protein synthesis in *E. coli*.<sup>160</sup> The hypothesis behind this observation was that the phenanthropiperidines were exerting their activity at the 80S ribosomal subunit, thus explaining why they were inactive in prokaryotic systems. To confirm this, a study explored the activity of three

phenanthropiperidines in *S. cerevisiae* and *E. coli*.<sup>193</sup> The activity mirrored that of the previous studies with cryptopleurine being the most potent. Ribosomal inhibition studies clearly showed that the phenanthropiperidines inhibited protein synthesis, with a noticeable preference for yeast cytoplasmic ribosomes over mitochondrial and *E. coli* ribosomes. Cryptopleurine was also shown to irreversibly inhibit ribosomal protein synthesis.

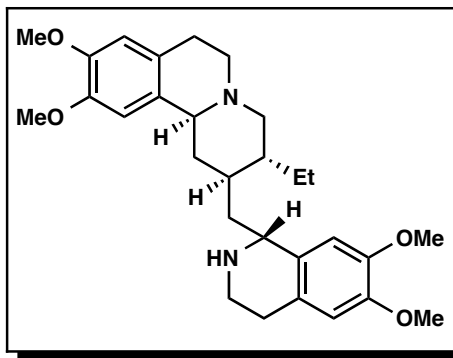
**Table 2-7.** Inhibition of protein, RNA and DNA synthesis

| entry | reference | model                                       | compound       | IC <sub>50</sub> (nM) |         |      |
|-------|-----------|---|----------------|-----------------------|---------|------|
|       |           |   |                | PS                    | RS      | DS   |
| 1     | 157       | Ehrlich ascites-tumor cells                 | cryptopleurine | 20                    | >100000 | –    |
|       |           | Ehrlich ascites-tumor cells                 | tylocrebrine   | 200                   | >100000 | –    |
|       |           | Ehrlich ascites-tumor cells                 | tylophorine    | 1000                  | >100000 | –    |
| 2     | 152       | HepG2 cells                                 | <i>E. coli</i> | cryptopleurine        | >10000  | –    |
|       |           |   | antofine       | 12                    | > 50    | 8    |
|       |           |   | ficuseptine    | 2500                  | 2500    | 1000 |
|       |           |   | cryptopleurine | <1000; >100000        | –       | –    |
| 3     | 189       | <i>S. cerevisiae</i> ; <i>E. coli</i>       | tylocrebrine   | 1000; >100000         | –       | –    |
|       |           |   | tylophorine    | 20000; >100000        | –       | –    |
|       |           |   | tylocrebrine   | 50                    | 300     | 60   |
| 4     | 190       | HeLa cells                                  | tylocrebrine   | 50                    | 300     | 60   |
|       |           | reticulocytes                               | tylocrebrine   | < 1000                | –       | –    |
| 5     | 194       | reticulocytes; reticulocyte lysate          | cryptopleurine | 150; 700              | –       | –    |
|       |           |   | tylocrebrine   | 300; 800              | –       | –    |
| 6     | 192       | <i>S. cerevisiae</i> ; cry mutant ribosomes | cryptopleurine | 180; 18000            | –       | –    |
| 7     | 196       | CHO cell wild type; Emt <sup>R</sup> mutant | cryptopleurine | 330; 660              | –       | –    |
|       |           |   | tylocrebrine   | 400; 1800             | –       | –    |

Diving deeper into the mechanism, Huang *et al.* examined tylocrebrine and its effects on HeLa cell and rabbit reticulocytes; a cell free model for protein biosynthesis.<sup>194</sup> Tylocrebrine, like cryptopleurine, irreversibly inhibited protein biosynthesis. In a more

recent study (-)-antofine, ficuseptice C, and DCB-3503 were also shown to dose-dependently inhibit protein biosynthesis in HeLa, HepG2, and/or Panc-1 cells.<sup>165,173</sup> Several phenanthropiperidines have been studied and corroborate those results showing that inhibition takes place during the translocation step of chain elongation.<sup>194-197</sup> When tylocrebrine was compared with a small collection of emetine-like alkaloids (Figure 2-8), its ameobocidal activity closely correlated with the activity of compounds that inhibited cell-free protein synthesis to the same extent.<sup>198</sup>

The comparison to emetine and the *Tylophora* alkaloids was not just coincidence. Emetine's natural source, ipecacuanha, has been used in traditional medicine, like *T. indica*, as natural emetics. There is also some speculation that due to their structural similarity they may have the same binding site on the ribosome.<sup>199</sup>



**Figure 2-8.** Structure of emetine.

Mounting evidence for ribosomal inhibition emerged from the identification of the *cry* gene that conferred resistance in *S. cerevisiae*.<sup>196</sup> Ribosomes isolated from the *cry* mutants required 100 fold more cryptopleurine to induce the same inhibitory effects. It was found that the normal (uninhibited) activity of the 40S ribosomal subunits in *cry*

mutants was impaired, revealing the biological outcome of the mutation.<sup>196,197</sup> This, however, does not clarify ribosomal subunit selectivity of these alkaloids. Genetic studies found that cryptopleurine (Cry<sup>R</sup>) and tylocrebrine (Tyl<sup>R</sup>) resistant CHO cells had a similar cross-resistance profile to emetine-resistant (Emt<sup>R</sup>) mutants.<sup>200</sup> Emt<sup>R</sup> cell's resistance could be traced back to a modified 40S ribosomal subunit. Since emetine is structurally similar to the phenanthropiperidines, it was deduced that the protein synthesis inhibitors shared a common binding site.<sup>199,200</sup> The proposed shared pharmacophore of emetine and cryptopleurine was not predictive of biological activity, as synthetic analogs with the proposed essential structural features were not active.<sup>201</sup>

Dolz *et al.* provided the evidence for ribosomal binding in 1982.<sup>202,203</sup> Displacement assays with tritiated cryptopleurine confirmed the existence of a single high-affinity binding site on both the 40S and 80S subunits. These sites are also shared with tylocrebrine and tylophorine, but distinct from emetine and tubulosine. Emetine and tubulosine only displaced cryptopleurine at very high concentrations, suggesting a separate binding site. Low-affinity interactions also exist on the 40S, 60S, and 80S subunits explaining cryptopleurine's peptide bond formation vs. translocation effects at high concentrations.

Another way the phenanthropiperidines exert their anti-proliferative effects is the inhibition of RNA and DNA synthesis. Tylocrebrine inhibits those two processes in HeLa cells (entry 4).<sup>194</sup> Antofine and DCB-3503 have also been shown to simultaneously inhibit protein and DNA synthesis (entry 2).<sup>165,173</sup> This is not surprising, because the pathways are interdependent, and inhibiting protein synthesis often inhibits DNA

synthesis as well. Because of this relationship, the phenanthropiperidines effects on DNA synthesis may be a minor mechanism of action and ribosomal inhibition the major one.

This evidence not only provides a mechanism of action, but also a specific molecular target. However, there is concern that targeting such an integral function could have undesired side effects. Recently, more evidence has emerged that selectivity can be obtained with protein synthesis inhibitors.<sup>204</sup> Protein synthesis that is unregulated is a major contributing factor in tumorigenesis<sup>205</sup> and metastatic progression.<sup>206</sup> Clinical studies of inhibitors, such as homoharringtonine and ET 743, have been performed, but are beset by cardiovascular complications, hypertension, ventricular tachycardia, atrial irritability, ST-T wave changes, and premature ventricular contractions.<sup>207-209</sup> Protein synthesis inhibitors have also suffered from other side effects such as memory loss and impaired motor skills.<sup>210,211</sup> Given these adverse effects, there is still significant interest in developing selective protein synthesis inhibitors with fewer side effects.<sup>204</sup>

Cheng *et al.* recently discovered that DCB-3503 preferentially down-regulated several key regulatory proteins such as cyclin D1, survivin,  $\beta$ -catenin, p53, and p21.<sup>165</sup> Although DCB-3503 suppressed global protein synthesis, the pro-oncogenic and pro-survival proteins were effected the greatest due to their short half-lives. Even though DCB-3503 affected protein synthesis, its effects are thought to be distinct from other protein synthesis inhibitors such as rapamycin.

The evidence presented in the last few paragraphs suggests that the phenanthropiperidines exert their cytotoxic effects primarily, although not exclusively, through protein biosynthesis inhibition. As shown through the tritiated cryptopleurine experiments,<sup>202</sup> the plausible molecular target is the 40S ribosomal subunit.



### 2.1.5.2.2 NF- $\kappa$ B and other regulatory pathways

A strong link between cancer and inflammation has emerged in the past decade and it has been recognized that the two are integrated at each stage of tumor development.<sup>212</sup> This has led to insights into the phenanthropiperidine's pharmacological effects when put side by side with their enveloping effects on the activity and expression levels of key regulatory proteins. Those effects are presented in Table 2-8.

NF- $\kappa$ B signaling pathways are an integral part of normal cell growth and immunological functions.<sup>213-215</sup> Gao *et al.* reported that tylophorine and DCB-3503 inhibited NF- $\kappa$ B-promoted transcription ( $IC_{50}$  = 30 and 100 nM respectively) and AP-1 and CRE, to a lesser extent.<sup>158</sup> DCB-3503 was chosen for further study because of its superior activity *in vivo*.<sup>163,168</sup> After a through investigation, it was concluded NF- $\kappa$ B inhibition resulted from increased proteasome-mediated degradation of nuclear phosphorylated p65 via an upstream down-regulation of nuclear IKK $\alpha$  and IKK inhibition.<sup>163</sup>

Tylophorine was also found to reduce TNF $\alpha$ , iNOS, and COX-2 production at 3-10  $\mu$ M without causing NF- $\kappa$ B inhibition in LPS/IFN $\gamma$ -induced macrophages.<sup>216</sup> AP-1 activation was also significantly inhibited.<sup>216</sup> This was accounted for by measuring the levels of upstream signaling factors MEKK1, Akt, and c-Jun. Akt activation counteracted the effect of tylophorine inhibition of MEKK1 activation, which normally inhibits NF- $\kappa$ B. Cryptopleurine dose dependently inhibited the TNF-induced expression of NF- $\kappa$ B.<sup>217</sup> Cryptopleurine also dose dependently inhibited the TNF-induced degradation and phosphorylation of I $\kappa$ B $\alpha$ .<sup>217</sup> These results suggest that cryptopleurine prevents TNF-

induced I $\kappa$ B $\alpha$  degradation through inhibition of I $\kappa$ B $\alpha$  phosphorylation, therefore interfering in the signaling cascade leading to NF- $\kappa$ B activation.

Besides NF- $\kappa$ B inhibition, several phenanthropiperidines are capable of down-regulating a number of other regulatory proteins. Boehmeriasin A suppresses the expression of cyclins D1 and E2.<sup>218</sup> Cyclin D1 is also down-regulated by tylophorine and DCB-3503 in a manner that is proportional to each compound's growth inhibition. DCB-3503 suppresses some key regulatory proteins as a downstream consequence of protein synthesis inhibition, such as cyclin D1, CDK4, cyclin B1, survivin,  $\beta$ -catenin, p53, and p21.<sup>163,165</sup> DCB-3503 dramatically reduced levels of cyclin D1 in multiple cells. This is clinically significant because cyclin D1 is an important cell cycle regulator and is frequently overexpressed in cancers and associated with oncogenicity.<sup>178</sup> On another note, unmitigated cell division in cancer could potentially be corrected by cyclin dependent kinases (CDK's), which govern cell cycle progression.<sup>219-222</sup>

**Table 2-8.** Effect on activity and expression levels of key regulatory proteins by phenanthropiperidines

| compound                | cell model                          | activity   | expression  | reference |
|-------------------------|-------------------------------------|--|---|-----------|
| tylophorine             | HepG2                               | ↓ NF-κB > AP-1, CRE<br>no effect on ERK 1/2          | ↓ nuclear IKKα, ↓ iNOS  | 156       |
|                         | LPS/IFN $\gamma$ -induced RAW 264.7 | ↓ AP-1, ↓ MEKK1, ↑ Akt<br>no effect on NF-κB         | ↓ iNOS, ↓ TNF $\alpha$ , ↓ COX-2  | 186, 212  |
| tylophorinidine         | HepG2                               | ↓ NF-κB > AP-1, CRE                                  | ↓ cyclin D1   | 165       |
|                         | HepG2                               | ↓ NF-κB > AP-1, CRE<br>no effect on ERK 1/2          | ↓ cyclin D1, ↓ CDK4, ↓ cyclin B1, ↓ survivin,<br>↓ β-catenin, ↓ p53, ↓ p21, ↓ TNF $\alpha$ , ↓ IL-1 $\beta$ , ↓ IL-12<br>↓ IL-6, ↓ MCP-1, ↓ iNOS, ↓ COX-2 | 156, 162  |
| DCB-3503                | HeLa                                | -  | ↓ cyclin D1, ↓ β-catenin, ↓ survivin  | 162       |
|                         | Huh7, MCF-7                         | -  | ↓ cyclin D1   | 162       |
|                         | Panc-1                              | ↓ NF-κB > AP-1, CRE, SRE<br>↓ IKK, ↓ IκB $\alpha$    | ↓ nuclear phosphorylated p65, ↓ IκB $\alpha$ , ↓ IKK $\alpha$<br>↓ cyclin D1, ↓ CDK4, ↓ cyclin B1   | 160       |
|                         | serum/joint tissue (DBA/1J mice)    | -  | ↓ TNF $\alpha$ , ↓ IL-1 $\beta$   | 161       |
|                         | bone marrow derived dendritic cells | -  | ↓ TNF $\alpha$ , ↓ IL-6, ↓ IL-12, ↓ MCP-1   | 161       |
| boehmeriasin A          | AGS                                 | ↓ HRE, ↓ HIF-1 $\alpha$ , no effect on HIF-1 $\beta$ | ↓ VEGF  | 225       |
|                         | MDA-MB-231                          | -  | ↓ cyclin D1, ↓ cyclin E2  | 214       |
| 7-methoxycryptopleurine | LPS/IFN $\gamma$ -induced RAW 264.7 | -  | ↓ iNOS, ↓ COX-2   | 186       |
| antofine                | LPS-induced RAW 264.7               | -  | ↓ iNOS  | 219       |

AP = activator protein; CDK = cyclin dependent kinase; COX = cyclooxygenase; CRE = camp response element; ERK = extracellular-signal-regulated protein kinase; HIF = hypoxia-inducible factor; HRE = hormone response element; IFN = interferon; IKK = IκB kinase; IL = interleukin; iNOS = inducible nitric-oxide synthase; LPS = lipopolysaccharide; MCP = monocyte chemotactic protein; MEKK = mitogen-activated protein/extracellular signal-regulated protein kinase; NF = nuclear factor; SRE = sreum response element; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor

Two inflammatory kinases were down-regulated by DCB-3503 at the systemic and local level, TNF $\alpha$  and IL-1 $\beta$ .<sup>164</sup> Immune-cell production of key inflammatory signaling molecules (IL-12, IL-6, MCP-1, iNOS, and COX-2) were also impaired by DCB-3503.

Nitric Oxide (NO) production was inhibited in LPS-stimulated macrophages by antofine, tylophorine, and several of their analogs. This appears to be a direct consequence of inhibiting iNOS expression.<sup>189,223</sup> Yang *et al.* demonstrated that the phenanthropiperidine's anti-cancer and anti-inflammatory activities are interrelated if not mechanistically the same by showing that NO-inhibition was proportionate with HONE-1 and NUGC-3 cell growth.<sup>189</sup>

The complex networks of biochemical processes within these pathways are interlaced with feedback loops, other regulatory pathways and other regulatory events that make the search for molecular targets an immense challenge.

#### **2.1.5.2.3 Apoptosis and cell cycle arrest**

Protein biosynthesis inhibitors will inherently suppress cell growth, but are not necessarily cytotoxic. Even though cytostatic agents can treat malignancies by subverting the accelerated proliferation in neoplastic growth, cancer cell death or differentiation is required for complete remission. An alkaloidal extract of *T. indica*, containing mainly tylophorine, tylophorinine, and tylophorinidine, completely inhibited cell growth in K562 (human erythroleukemic) cells at 0.1  $\mu\text{g}/\text{mL}$ .<sup>224</sup> When the concentration was increased by ten-fold, the cells underwent apoptosis. While apoptotic agents are ideal for chemotherapeutics, it is questionable whether the concentrations used here are therapeutically relevant. Additionally, since the alkaloidal mixture was used, a synergistic effect cannot be ruled out. The analysis was simplified by Lee *et al.* using antofine as a single component.<sup>225</sup> Potent inhibition of growth and colony formation was observed in A549 and Col2 cancer cells, however no evidence of apoptosis and necrosis was observed at low concentrations. This evidence suggests that antofine alone is primarily cytostatic. However, in HCT-116 cells stimulated with  $\text{TNF}\alpha$ , antofine enhanced  $\text{TNF}\alpha$  induced apoptosis by approximately 29%.<sup>226</sup>

**Table 2-9.** Apoptosis and cell cycle arrest by phenanthropiperidine alkaloids

| entry | reference | cell line                 | compound                 | concentration | result as cytostatic concentrations            |
|-------|-----------|---------------------------|--------------------------|---------------|--|
| 1     | 220       | K562                      | <i>T. indica extract</i> | 0.1 µg/mL     | no apoptosis                                   |
|       |           |                           |                          | 1 µg/mL       | apoptosis                                      |
| 2     | 221       | A549, Col2                | antofine                 | <30 nM        | no apoptosis                                   |
|       |           | Col2                      | antofine                 | 0.1 nM        | G2/M phase arrest; morphological changes       |
| 3     | 156       | KB                        | tylophorine, DCB-3503    | 0.03-1 µM     | S phase accumulation<br>no DNA damage          |
|       |           | HepG2                     | tylophorine, DCB-3503    | 0.3-3 µM      | sustained growth inhibition                    |
| 4     | 223       | HONE-1<br>NUGC-3<br>HepG2 | tylophorine              | 2 µM          | sustained growth inhibition<br>G1 phase arrest |
| 5     | 214       | MDA-MB-231                | boehmeriasin A           | 19 nM         | G1 phase arrest, no apoptosis                  |

DCB-3503 and tylophorine did not cause DNA damage. This was indicated by p53 not being induced during treatment.<sup>158</sup> In fact, DCB-3503 did not even induce apoptosis or necrosis at 3 µM concentrations. As a follow up, apoptosis was observed in Panc-1 cells when treated with DCB-3503 for two-generation times, but not one.<sup>163</sup> When HepG2 cells were treated with DCB-3503 and tylophorine for 8 days, the result was complete and sustained growth inhibition.<sup>158</sup> This activity was also observed for tylophorine treated HONE-1 and NUGC-3 cells.<sup>227</sup> These long lasting effects are notable, considering these alkaloids induce irreversible protein synthesis inhibition.

In concentrations as low as 0.1 nM, antofine induced morphological changes and G2/M cell cycle arrest in Col2 cells.<sup>224</sup> KB cells accumulated in the S phase when treated with both tylophorine and DCB-3503, whereas in HepG2 and Panc-1 cells did not accumulate at any particular phase.<sup>158,163</sup> G1-phase arrest was induced by tylophorine in HepG2, HONE-1 and NUGC-3 cells. The G1-phase arrest induced by tylophorine was linked to a suppression of cyclin A2 expression.<sup>175</sup> Boehmeriasin A also induced G1 cell cycle arrest, but not apoptosis, in the multidrug resistant MDA-MB-231 cell line.<sup>218</sup> In

this study, cyclins D1 and E2 were underexpressed in the treated cells. As stated earlier, CDK's modulate the cell progression cycle. Modulation of CDK's could potentially correct the faulty cellular pathways that lead to unregulated cell division in cancer. The data presented in this section provides an insight into the phenanthropiperidines as cytostatic and not apoptotic agents.

#### **2.1.5.2.4 Cell differentiation**

Given that the phenanthropiperidines inhibit protein synthesis inhibition, it is not surprising that they elicit phenotypic changes in transformed cells. The possibility that tylophorine and DCB-3503 induced cell differentiation was investigated through monitoring the expression levels of two tumor biomarkers, alpha-fetoprotein (AFP) and albumin.<sup>158</sup> Treatment of HepG2 cells with tylophorine or DCB-3503 resulted in a suppression of AFP expression and an increase in albumin expression, consistent with cell differentiation. DCB-3503 was also found to induce cell differentiation in Panc-1 cells.<sup>165</sup> Boehmeriasin A has also been shown to cause differentiation through alterations in cell morphology, lipid droplet accumulation, and gene expression.<sup>176,218,227</sup>

#### **2.1.5.2.5 Angiogenesis**

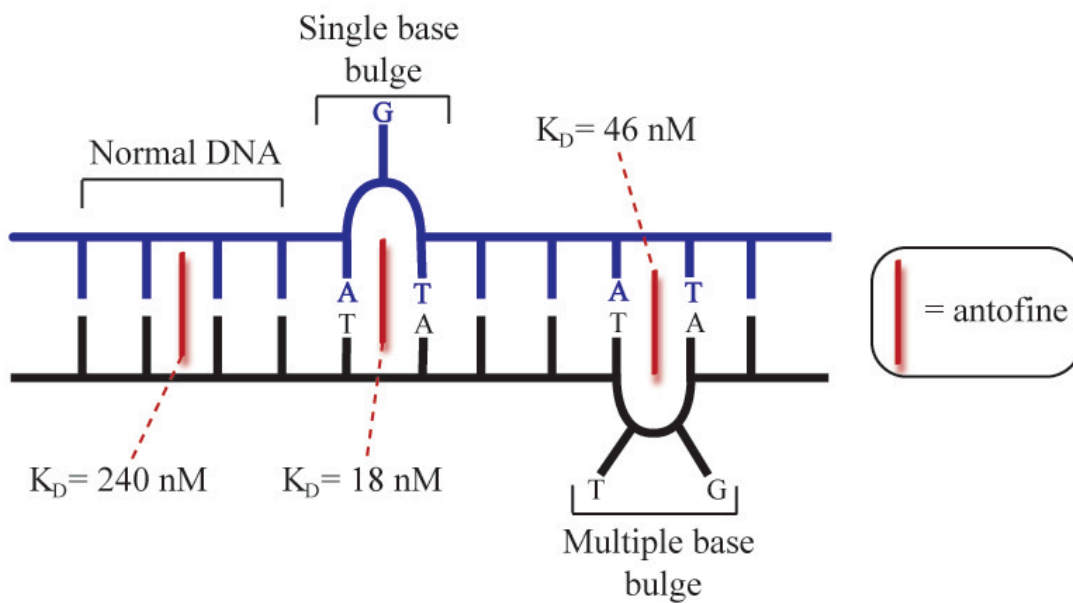
Another hallmark of cancer is “sustained angiogenesis.”<sup>228</sup> Elevated metabolic demand in cells, such as those in many types of tumors, require sufficient vasculature to sustain their aerobic demands. Slow growing tumors also require an active blood supply for their basic existence. This is accomplished by release of growth factors such as VEGF, which is triggered by hypoxia. Thus, targeting angiogenesis is a highly effective way to suppress

tumor growth. Cryptopleurine and 15*R*-hydroxycryptopleurine effect hypoxia inducible factor-1 (HIF-1), a key regulator of a cell's response to hypoxia.<sup>229</sup> They were found to inhibit HIF-1 mediated gene expression under anaerobic conditions with IC<sub>50</sub>'s of 8.7 and 48.1 nM respectively.<sup>230,231</sup> Unfortunately, this activity suffered *in vitro* due to cryptopleurine's poor solubility.<sup>232</sup> However, a number of more soluble seco-analogs did inhibit angiogenesis in rat aorta blood vessel fragments in low micromolar concentrations.<sup>170,232</sup>

#### **2.1.5.2.6 DNA and RNA intercalation**

DNA and RNA have also been investigated as targets for the phenanthropiperidines. Antofine's associative properties were studied against a library of DNA oligonucleotides containing a variety of secondary structures (hairpin bends, bulges etc.).<sup>171</sup> A clear preference for duplex oligonucleotides was shown by antofine as measured by dissociation from and stability of the DNA complex-alkaloid complex. Antofine also binds with moderate selectivity for DNA bulges in a 1:1 stoichiometry and slight sequence selectivity (Figure 2-9). This is significant because these abnormalities are implicated in numerous diseases such as HIV, Huntington's disease and Friedreich's ataxia.<sup>233,234</sup> Docking studies with bulged DNA add credence to the hypothesis that antofine's association with bulged DNA is intercalative. It is worth mentioning that tylophorine does not induce DNA damage, however its affinity for DNA is yet to be

investigated. Recently, it was shown that DCB-3503's antiproliferative effects are independent of DNA binding.<sup>165</sup>



**Figure 2-9.** Antofine's affinity for DNA.

Along with DNA binding, antofine has shown affinity for RNA of the Tobacco Mosaic Virus (TMV).<sup>235</sup> This RNA motif contains hairpin loop structures with several bulges. Antofine binds TMV RNA with a  $K_D$  of 9 nM and a 1:25 stoichiometry. Antofine's affinity for DNA and RNA needs to be considered in discussions about its mechanism of action and needs more investigation. This also needs to be investigated for other members of this class and correlated with their antiproliferative activities. Given the planarity of the phenanthropiperidines, the question of intercalation is obvious and needs to be addressed.



### 2.1.5.2.7 Other targets

Several other molecular targets have been proposed, other than the 40S ribosomal subunit, DNA, and RNA. Tylophorinidine has been observed to inhibit thymidylate synthase and dihydrofolate reductase at higher concentrations ( $IC_{50} > 30 \mu\text{M}$ ). Using these targets to explain the phenanthropiperidine's anticancer activity is uncalled for, since the phenanthropiperidines exhibit activity in the low nanomolar range.

### 2.1.5.3 Anti-inflammatory/immune activity

The bases for anti-inflammatory/immunosuppressive effects are not straightforward. *T. indica* plant extracts were clearly shown to suppress the inflammatory response in cell, animal, and human models, as discussed earlier. Keeping this in mind, there has been significant effort dedicated to investigating the anti-inflammatory properties of the pure alkaloids. Tylophorine, the primary active component of *T. indica*, reduced immunopathological and inflammatory response *in vitro* and *in vivo*.<sup>236,237</sup> Due to its improved *in vivo* activity, DCB-3503 has been studied more extensively for its anti-inflammatory activity. DCB-3503 outperformed tylophorine in animal studies, presumably due to its improved pharmacokinetics and/or solubility, despite its inferior NF- $\kappa$ B activity.<sup>158</sup> Mice injected with collagen or lipopolysaccharide (LPS) to induce arthritis were treated with DCB-3503 and monitored for signs of efficacy. The development, onset, and severity of LPS and collagen-induced arthritis were decreased with no signs of toxicity. TNF $\alpha$  and IL-1 $\beta$ , two important pro-inflammatory cytokines,

were down regulated at the systemic and local level. Furthermore, immune-cell production of key inflammatory signaling molecules, such as  $\text{TNF}\alpha$ , IL-12, IL-6, MCP-1, iNOS and COX-2, was significantly impaired.

With evidence of DCB-3503's immunosuppressive effects, the compound was also investigated for systemic lupus erythematosus (SLE) in a phenotypic murine model.<sup>162</sup> The inflammatory skin lesions were dramatically reduced with no signs of hematologic or hepatic toxicity at therapeutic doses. Serum levels of IgG, the IgG1 isotype, in particular-and autoantibodies were suppressed by DCB-3503. This suggests that curbing the Th2-driven immune response is responsible for DCB-3503's therapeutic effect. In spite of these immunosuppressant properties, there was little improvement of histologic kidney disease.

Inhibition of NF- $\kappa$ B transcription is a plausible explanation for the extensive biological effects, although this view may be a bit simplistic. As noted earlier, early work found that tylophorine significantly reduced  $\text{TNF}\alpha$ , iNOS and COX-2 production at 3-10  $\mu\text{M}$  without causing NF- $\kappa$ B inhibition, while inhibiting AP1 activation.<sup>216</sup> This was accounted for by measuring the levels of upstream signaling factors MEKK1, Akt, and c-Jun. Akt activation counteracted the effect of tylophorine inhibition of MEEK1 activation, which normally inhibits NF- $\kappa$ B. The relation of these events to the attenuation of inflammatory response is not immediately obvious, the molecular basis for the response remains to be elucidated.

#### 2.1.5.4 Miscellaneous activities

In addition to the phenanthropiperidine's anti-cancer and anti-inflammatory properties, they have been investigated for activity in several other biological systems. Cryptopleurine had colchicine-like c-mitotic activity in the root tips of germinating onion seeds.<sup>238</sup> However, cryptopleurine's activity did not resemble that of colchicine in oyster embryos or yeast, even though it did inhibit their growth.<sup>239</sup> Anti-fungal,<sup>240</sup> amoebicidal,<sup>198,241</sup> insecticidal,<sup>242</sup> and anti-feedant<sup>243</sup> properties have also been reported for cryptopleurine and some closely related analogs.

The phenanthropiperidines have also been reported to have anti-viral properties. Viral growth was stunted when kidney cells were treated with cryptopleurine and subsequently inoculated with herpes virus hominis.<sup>244</sup> However, no anti-viral activity against coxsackie B-5 or polio-type I viruses was observed. Antofine exhibited anti-TMV activity in the low micromolar range, presumably due to its affinity for RNA.<sup>245</sup> Tylophorine (racemic, *R*, and *S*), (±)-antofine, and (±)-deoxytylophorine also exhibited anti-TMV activity. A series of tylophorine salt derivatives were prepared with the goal of improving solubility and stability.<sup>246</sup> These analogs were not only more stable and soluble; they also had improved *in vitro* and *in vivo* anti-TMV activity, surpassing that of commercial Ningnanmycin. Tylophorine and 7-methoxycryptopleurine were also found to possess potent anti-TGEV and anti-SARS CoV agents with EC<sub>50</sub> values in the low nanomolar range, on par with current therapeutics.<sup>247,248</sup>

### **2.1.5.5 Summary of biological activity**

The extraordinary activities of the phenanthropiperidines are hard to ignore, but have yet to be clinically relevant. In the past 10-15 years there has been a considerable effort to assess their therapeutic potential as both anti-cancer and anti-inflammatory/immune agents. The exact mechanisms by which the phenanthropiperidines exert their therapeutic effects are difficult to ascertain, because data are collected from different assay, cellular, and animal models. Currently, protein biosynthesis inhibition and modulation of key transcription factors are the most well supported mechanisms and can account for the extensive anti-cancer and anti-inflammatory/immune effects of the phenanthropiperidines. However, it is becoming more likely that the alkaloids act on more than one molecular target. For example, the phenanthropiperidines have affinity for the 40S ribosomal subunit, DNA, and RNA. Thus, the pharmacology of these alkaloids is not only complex but also multifaceted and will require further investigations.

### **2.1.6 Barriers impeding clinical success**

The extraordinary activity the phenanthropiperidines possess *in vitro*, has not translated to *in vivo* animal models. The NCI has conducted multiple *in vivo* studies with tylocrebrine, tylophorine, and tylophorinine. It was found that all of these alkaloids provided 150% life extension in L1210 leukemia model, the most responsive tumor model. However, other tumor models were much less responsive (adenocarcinoma 755 and sarcoma 180). The discrepancy between *in vivo/in vitro* activity is a difficult question

to address, and likely has many contributing factors that are just beginning to be understood.

Despite the similar activities *in vitro*, tylophorine and DCB-3503 behave remarkably different in animal studies.<sup>158</sup> The NCI scored both in the NCI hollow fiber assay with DCB-3503 outscoring tylophorine by a large margin (26 vs. 4). The compounds that score greater than 20 are generally considered for further animal studies.<sup>249</sup> It was proposed that this difference was due to drug delivery or stability. Both compounds were subsequently tested in a HepG2 xenograph murine model. DCB-3503 again outperformed tylophorine with superior anti-tumor activity.<sup>158</sup> This result could be explained from differing pharmacokinetic profiles, but no extensive pharmacokinetic studies have been reported. Pharmacokinetic profiles would be invaluable to understanding why such potent compounds fail to perform *in vivo*.

Another important consideration in the discussion of the phenanthropiperidines is the therapeutic window. Some toxicological data can be obtained from the NCI database, which reports the mortality in each model system. From this and other *in vivo* studies, it can be garnered that an effective dose can be reached without causing explicit toxic effects. In this respect, tylophorine has been studied most extensively. One study reported that when rats were treated orally with tylophorine (1.25-2.5 mg/kg/d) over 15 days no toxic effects were observed. However, elevated doses (35 mg/kg oral dose) were lethal to 50% of the rats.<sup>250</sup> In a different study, rats and hamsters treated with a 5 mg/kg oral dose of tylophorine was lethal and produced 50 and 80% mortality rates, respectively.<sup>241</sup> In a contrasting report (*R*)-tylophorine at a 500 mg/kg dose delivered *i.p.* was non-toxic to rats.<sup>174</sup> Because DCB-3503's preferential effect on proteins with short half-lives, it has

been hypothesized that the therapeutic index will be highly dependent on the dosing regimen.<sup>165</sup> This theory turned out to be valid, because DCB-3503 proved to be effective for treating cancer, lupus, and arthritis in mouse models when administered every 3 days without any obvious signs of toxicity.<sup>162-165</sup>

Another potential issue with the phenanthropiperidines is their neurological side effects. It was observed that tylophorine induced CNS depression (ptosis, sedation, decreased motor activity, and staggered gait) at high doses in rats.<sup>236</sup> This is relevant because tylocrebrine failed out of clinical trials due to adverse neurological side effects such as ataxia and disorientation.<sup>104</sup> In light of tylocrebrine's clinical failure and realizing that tylophorine (a regioisomer of tylocrebrine) would have similar physiochemical properties; it is clear why these side effects pose a major concern. It is well known that the degree of passive diffusion across the blood-brain barrier (BBB) correlates with the molecule's polar surface area (PSA), number of H-bond donors (HBD), cLogP, cLogD, and molecular weight.<sup>251</sup> Calculating these properties for tylophorine and tylocrebrine shows that each value falls well within the parameters for CNS drugs (Table 1-10). Protein synthesis inhibitors have shown to have adverse effects on motor control and memory, which is particularly concerning for the phenanthropiperidines.<sup>210,211</sup> A strategy to alleviate the CNS-side effects is to design analogs that make BBB permeation improbable, such as polar analogs.

**Table 2-10.** CNS drugs and tylocrebrine's physicochemical properties

| property              | top 25 CNS drugs<br>mean values | suggested limits | tylocrebrine |
|-----------------------|---------------------------------|------------------|--------------|
| PSA (Å <sup>2</sup> ) | 47                              | < 90             | 40           |
| hydrogen bond donors  | 0.8                             | < 3              | 0            |
| cLogP                 | 2.8                             | 2-5              | 4.3          |
| molecular weight      | 293                             | < 500            | 394          |

The last major concern I will discuss is the poor solubility of the phenanthropiperidines. The high lipophilicity of these molecules is the most likely the major contributing factor to their poor solubility. Fortunately, by mitigating the poor solubility via the addition of polar functional groups, we can also prevent BBB permeation. The planar structure of the phenanthropiperidines is another contributing factor to low solubility. A recent review shows that increasing the sp<sup>3</sup> character in drug candidates correlates with improved solubility, and therefore clinical success.<sup>252</sup> It is also not clear what structural modifications can be made to increase activity or improve solubility, because of the limited understanding of the SAR. Improving the aqueous solubility by the addition of a hydroxyl group in DCB-3503<sup>158</sup> and retaining most of the activity was a limited breakthrough since this compound is still expected to cross the BBB, because of its highly similar physicochemical properties compared to the parent compound. Another solubility enhancement occurred via the preparation of salt derivatives, but salt formation would not effect BBB penetration.<sup>246</sup> Other modifications

have been made that improved solubility (such as incorporation of heteroatoms into the E-ring), but resulted in a significant reduction of activity.<sup>117,188,192</sup> Although the hindrances to clinical success of the phenanthropiperidines seem daunting, there are many ideas that have yet to be explored and in those ideas may lie the answers to achieve the goal of clinical relevance.

### **2.1.6 Synthesis of phenanthropiperidine alkaloids**

Drug development is largely determined by the accessibility (synthetic, semi-synthetic, or biosynthetic) of the bioactive molecule. Without adequate supply, the most active drugs could not be developed for clinical use. With that being said, supply is not a limitation for preclinical investigations of the phenanthropiperidines. The phenanthropiperidines have reliable synthetic routes that for the most part have supplanted isolation. The phenanthropiperidines have attracted the attention of organic and medicinal chemists due to their unique structural features and extraordinary activity. This has provided a sustainable source of these alkaloids and given access to novel synthetic analogs with improved therapeutic potential.

Synthetic approaches to the phenanthropiperidines have improved tremendously over the years. Advances in transition metal catalysis and heterocycle synthesis have played a major role in this respect. As mentioned previously, the phenanthropiperidines have attracted the attention of organic and medicinal chemists alike. Tylophorine and cryptopleurine alone have over twenty distinct synthetic routes by which they can be prepared. Antofine is another popular target among synthetic and medicinal chemists



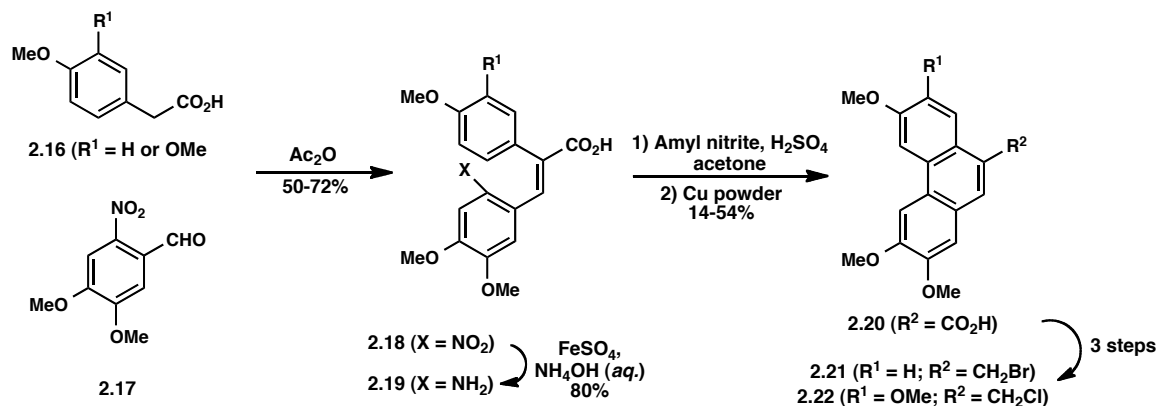
because of its potent cytotoxicity.

Even though synthetic endeavors have employed novel reactions, the typical synthesis generally follows one of two strategies. The first commonly employed strategy revolves around the synthesis of the phenanthrene system, followed by the synthesis of the indolizidine or quinolizidine portion of the molecule. The second is the reverse scenario of the previous strategy synthesizing the indolizidine or quinolizidine first and then followed by the synthesis of the phenanthrene portion. Although the total syntheses are beyond the scope of this work, the focus will be on the key steps in the synthesis of the quinolizidine/indolizidine scaffolds and the phenanthrene system with a brief detour into the very first synthetic endeavors.

### 2.1.6.1 First synthetic endeavors

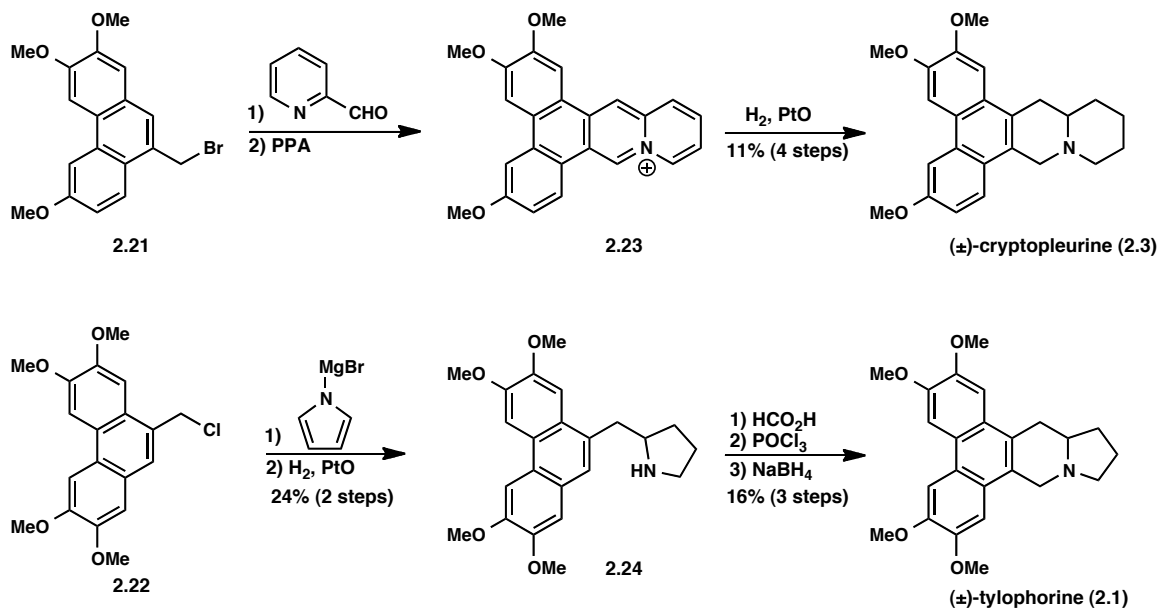
Just five years after cryptopleurine's structure was elucidated, the first synthesis was reported.<sup>253,254</sup> A synthesis of tylophorine followed closely behind, reported in 1961.<sup>113</sup> The delay in the synthesis of tylophorine, isolation reported in 1935, was due to

**Scheme 2-2.** First method to prepare phenanthrene system



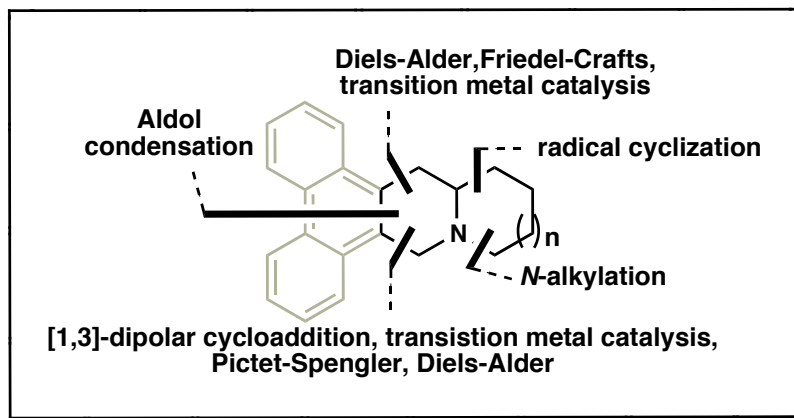
structural uncertainties. The methods used in the synthesis of cryptopleurine and tylophorine allowed for the synthesis of tylocrebrine, which was reported along with its isolation.<sup>121</sup> The synthesis commenced with the condensation of phenylacetic acid **2.16** and nitroveratraldehyde **2.17** (Scheme 2-2). The reduction of the nitro group using ferrous sulfate to the amine prepared the system for phenanthrene formation (**2.18** to **2.19**). The amine was converted to the diazonium salt, and subsequent treatment with copper mediated a radical decomposition of the diazo group and bond formation to furnish phenanthrene **2.20**. The acid was then reduced to the alcohol followed by conversion of the alcohol to the corresponding alkyl bromide **2.21** or the alkyl chloride **2.22**. As shown in Scheme 2-3 the alkyl bromide **2.21** was then used to *N*-alkylate picolinic aldehyde and cyclized in polyphosphoric acid (PPA) to give the fully oxidized pentacyclic system **2.23**. The final step in the synthesis of cryptopleurine (**2.3**) was the reduction of the fully oxidized system to the phenanthropiperidine natural product. Tylophorine (**2.1**) on the other hand was synthesized in a different manner (Scheme 2.3). The alkyl chloride was reacted with pyrrolmagnesium bromide and subsequently hydrogenated to the alkyl pyrrolidine **2.24**. The completion of the synthesis was achieved using a Bischler-Napieralski protocol. The synthesis of tylocrebrine was completed in a very similar manner.<sup>121</sup>

### Scheme 2-3. First syntheses of cryptopleurine and tylophorine



#### 2.1.6.2 Synthesis of indolizidine and quinolizidine scaffold

The discussion of the syntheses will start with the indolizidine and quinolizidine bicyclic structures due to the vast variety of strategies. In an attempt to breakdown/simplify the discussion; all of the strategies discussed will be based on reaction type (aldol, Pictet-Spengler, transition metal catalysis, Diels-Alder, dipolar cycloadditions, Friedel-Crafts, *N*-alkylation, and radical cyclizations).



**Figure 2-10.** Approaches toward the synthesis of quinolizidine/indolizidine.

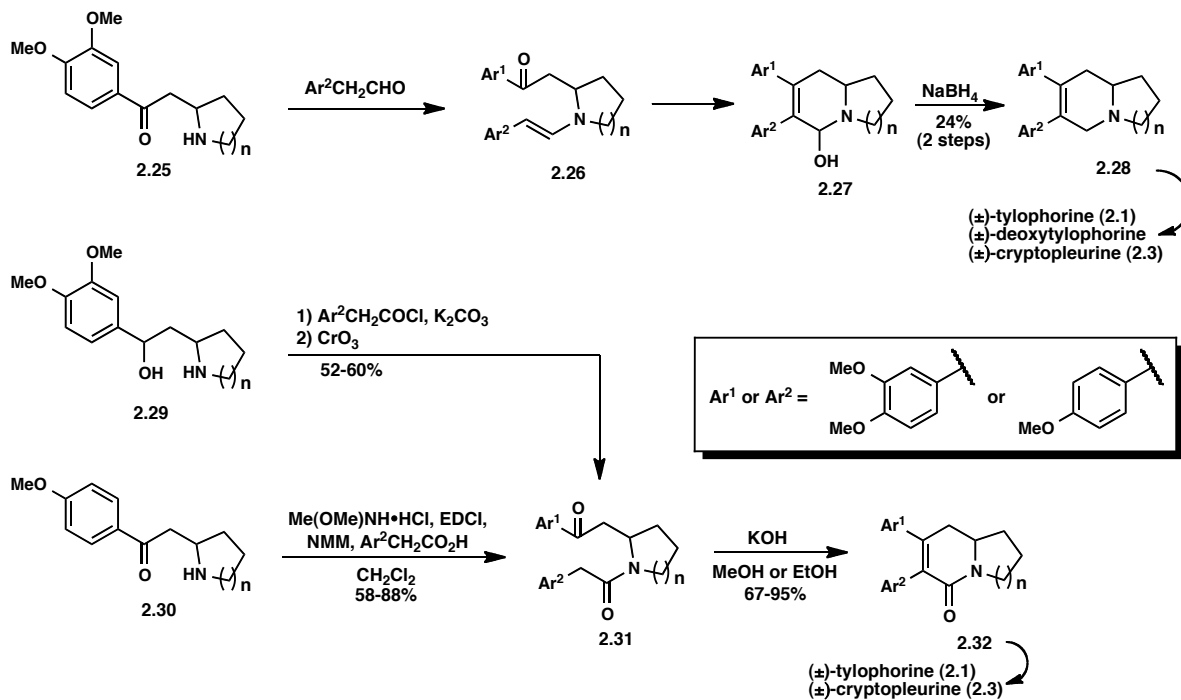
#### 2.1.6.2.1 Aldol reactions

The intramolecular aldol reaction has been used to form the piperidine portion of the phenanthropiperidines for quite some time.<sup>255-261</sup> This basic reaction allows for the synthesis of the natural products in a relatively small number of steps.

To this end, two aldol approaches have been developed, one of which is a biosynthetic approach (Scheme 2-4). The biosynthetic approach involves the condensation of amine **2.25** with the appropriate phenylacetaldehyde.<sup>136,257,261-266</sup> The enamine product **2.26** then spontaneously cyclizes to the bis-arylated indolizidine **2.27**. Intermediate **2.27** then is further reduced with sodium borohydride to give seco-analog **2.28**. This sets the stage for the final biaryl couplings to give the natural products. While this approach is quite concise, it suffers from poor yields. The other approach features the acylation of amines **2.29** or **2.30**.<sup>259-261,267-269</sup> The resultant diketone **2.31** is then treated with KOH in an alcoholic solvent to provide the seco-amide analog **2.32**. Just as before,

these are now set up for the final oxidative biaryl coupling to provide the natural products.

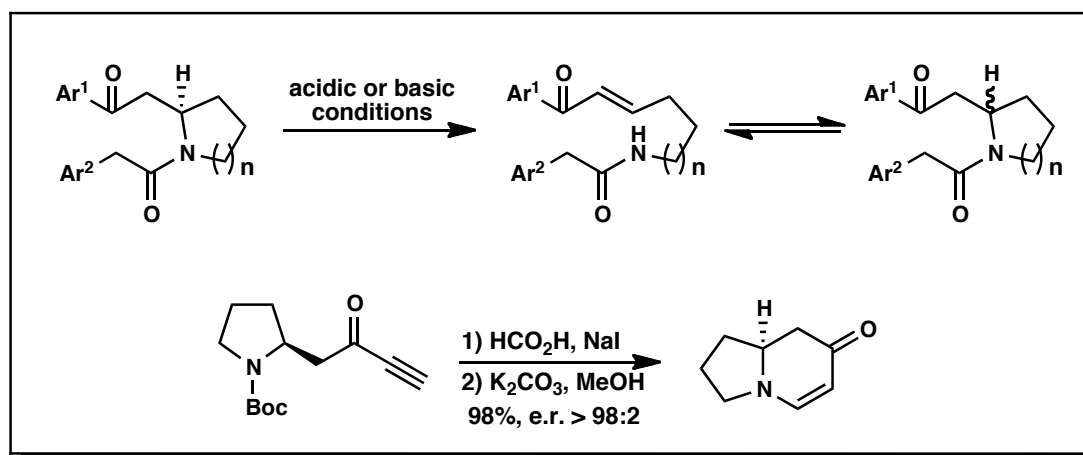
**Scheme 2-4.** Aldol reactions for the synthesis of the indolizidine/quinolizidine system



While the aldol reaction is a concise method for the construction of the phenanthropiperidines, the approach suffers from some limitations. The major limitation was found when enantiomerically pure starting material partially racemized when subjected to the reaction conditions.<sup>258,260</sup> Further investigations into the cause of the racemization, revealed that acidic removal of the Boc-protecting group (Boc-**2.30**) and the base-promoted aldol condensation (**2.31** to **2.32**) were the cause of the problem (Scheme 2-5).<sup>260</sup> It was then proposed that a *retro*-Michael process causes this type of

racemization. This racemization has limited the use of the aldol approach for the enantiospecific synthesis of the phenanthropiperidines. Fortunately, a solution was found for the acidic racemization process. A discovery that weak acids, such as formic acid ( $\text{HCO}_2\text{H}$ ), could mitigate such *retro*-Michael type racemization as well as be carried out at room temperature and open to air was very promising.<sup>47,270</sup> When these conditions were applied to (*S*)-*tert*-butyl 2-(2-oxobut-3-ynyl)pyrrolidine-1-carboxylate (Scheme 2-5), the racemization process to from the corresponding bicyclic enaminone was alleviated.<sup>47</sup> While this process has not been directly applied to the aldol reaction process, it could be one solution to the problems associated with the racemization process encountered with this concise synthetic aldol approach.

**Scheme 2-5.** Mechanism of racemization and possible solution

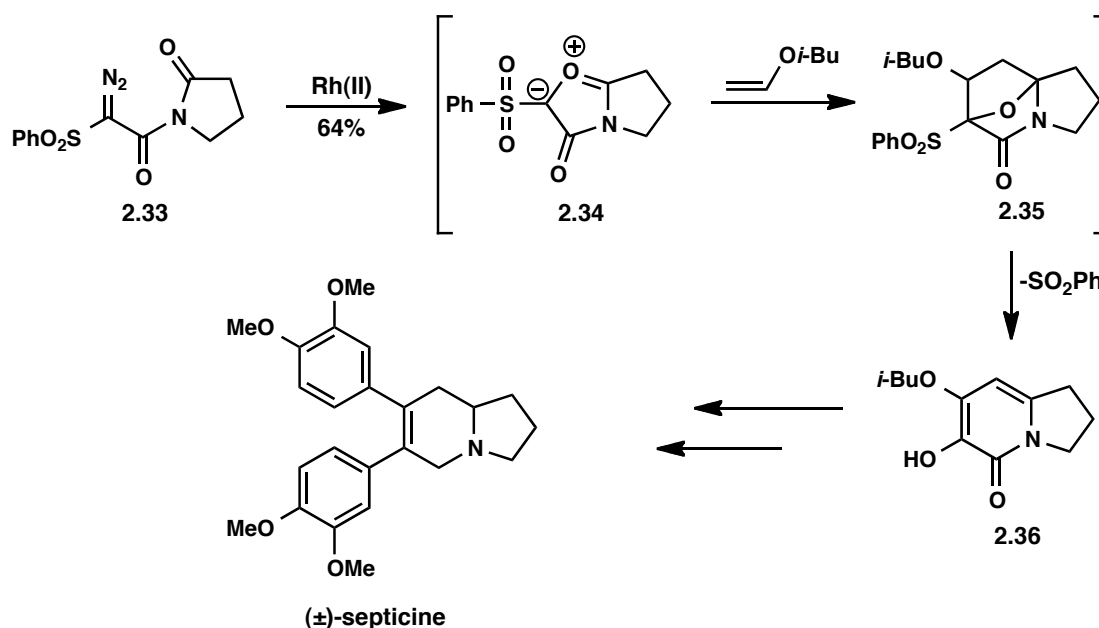


### 2.1.6.2.2 Dipolar cycloadditions

Dipolar cycloadditions are a class of pericyclic reactions that involve the cyclization of a dipole and a dipolarophile. These types of reactions are of high value, especially in the synthesis of 5-membered heterocycles.

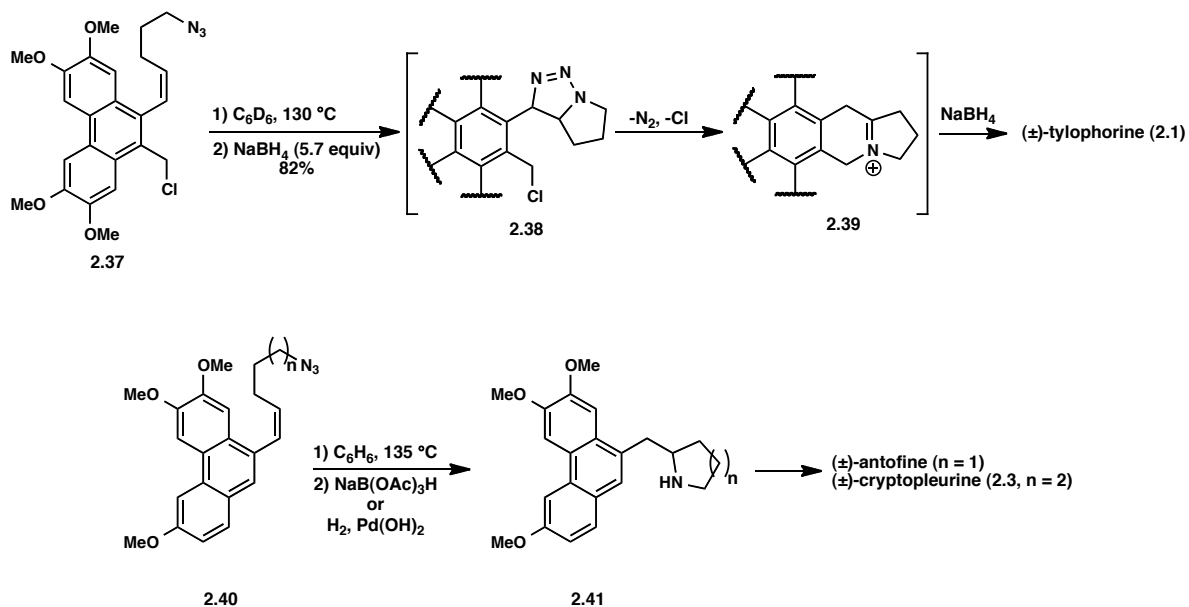
Padwa *et al.* have developed a Rh(II)-catalyzed [1,3]-dipolar cycloaddition for the synthesis of substituted dihydroindolizin-(1H)-one's (Scheme 2-6).<sup>271</sup> Treatment of pyrrolidine **2.33** with Rh(OAc)<sub>2</sub> generates the cyclic dipole **2.34** *in situ*, which reacts with alkenes to provide the bridged-bicyclic system **2.35**. The bridged-bicyclic system spontaneously decomposes via loss of SO<sub>2</sub>Ph to give the dihydroindolizin-(1H)-one **2.36**. This methodology was used to complete a formal synthesis of (±)-septicine.

**Scheme 2-6.** [1,3]-Dipolar cycloaddition cascade toward the formal synthesis of (±)-septicine



Pearson and Walavalkar took a strikingly different approach towards the synthesis of antofine and cryptopleurine (Scheme 2-7). While they used a [1,3]-dipolar cycloaddition, an azide was reacted with alkenes to form triazolines.<sup>272</sup> Azide **2.37** undergoes a cyclization with the pendant alkene upon heating to form the triazoline **2.38**. A second cyclization occurs when the triazoline collapses via loss of nitrogen and then an attack on the alkyl chloride provides intermediate **2.39**. Intermediate **2.39** is then reduced by NaBH<sub>4</sub> to complete the synthesis of tylophorine. The same approach was recently taken in the synthesis of (±)-cryptopleurine and (±)-antofine.<sup>273</sup>

**Scheme 2-7.** Azide mediated dipolar cycloadditions towards the synthesis of phenanthropiperidines



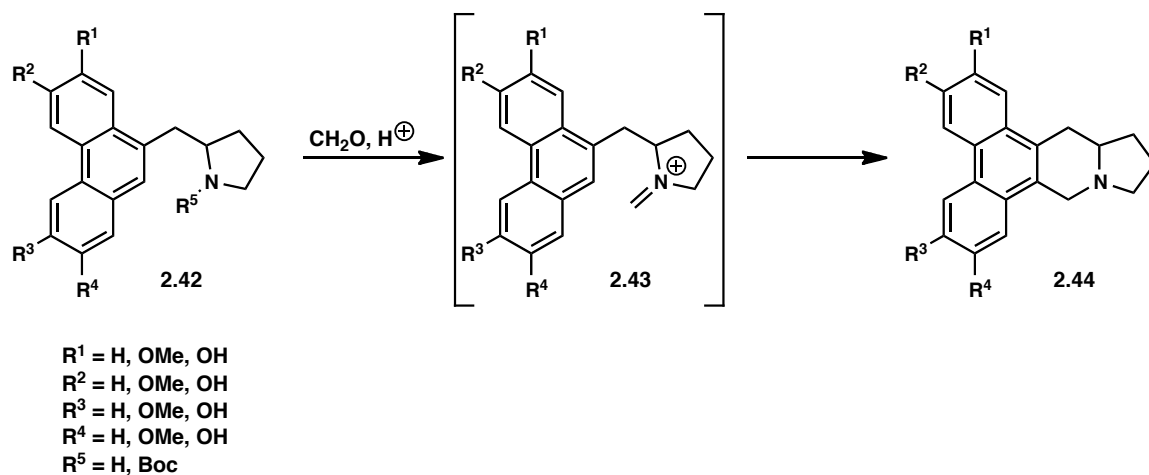


### 2.1.6.2.3 Pictet-Spengler and Friedel-Crafts reactions

The Pictet-Spengler and Friedel-Crafts reactions are somewhat related. They both alkylate an aryl ring under acidic conditions. Where they differ is the electrophile. In the case of the Pictet-Spengler reaction the electrophile is an iminium ion formed by the condensation of an amine with an aldehyde. The Friedel-Crafts reactions use alkyl halides or acid chlorides as the electrophiles. Both of these approaches lend themselves well to the synthesis of phenanthropiperidines.

The Pictet-Spengler reaction offers a direct approach towards the synthesis of phenanthropiperidines, and is now a very commonly used strategy. A general reaction scheme is shown below (Scheme 2-8). The pyrrolidine is treated with formaldehyde under acidic conditions to form the reactive iminium ion *in situ*, and if needed the nitrogen can be deprotected by an acid-labile protecting group as well under these conditions. The phenanthrene system then spontaneously reacts with the iminium ion to give the phenanthropiperidine. This strategy has been used to synthesize many of the natural products and natural product analogs.<sup>173,191,273-278</sup>

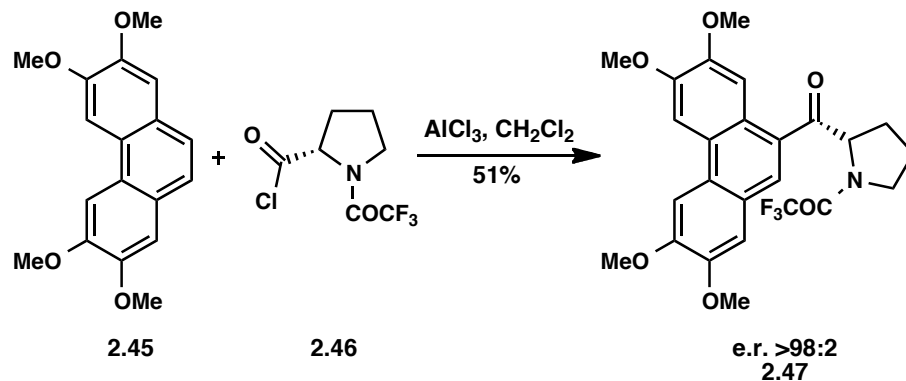
**Scheme 2-8.** Pictet-Spengler approach towards the synthesis of phenanthropiperidines



The direct Friedel-Crafts approach towards the synthesis of phenanthropiperidines is quite practical but has its drawbacks. The cyclization precursor is usually derived from an amino acid and the phenanthrene. During this chiral pool approach the amino acid chloride has a propensity to racemize.<sup>279</sup> To “muddy the waters” even more the wide range of optical rotations reported make it very difficult to deduce optical purity with any sort of confidence. Another drawback of this approach is that it is dependent on specific Lewis acids.<sup>116</sup> For example, the use of  $\text{AlCl}_3$  gave low yields and caused partial demethylation of the aromatic ethers; while the use of  $\text{SnCl}_4$  produced quantitative yields.<sup>168,280</sup> One major improvement is noteworthy, showing that an intermolecular Friedel-Crafts is possible without causing stereochemical degradation (Scheme 2-9). In this case the authors used trifluoroacetylpropyl chloride **2.46** and the symmetrical phenanthrene system **2.45** in the presence of  $\text{AlCl}_3$ . Given the difficulties determining

enantiomeric purity presented earlier, a lanthanoid chiral shift reagent was used to determine the enantiomeric ratio for **2.47**.<sup>276</sup>

**Scheme 2-9.** Friedel-Crafts approach without racemization



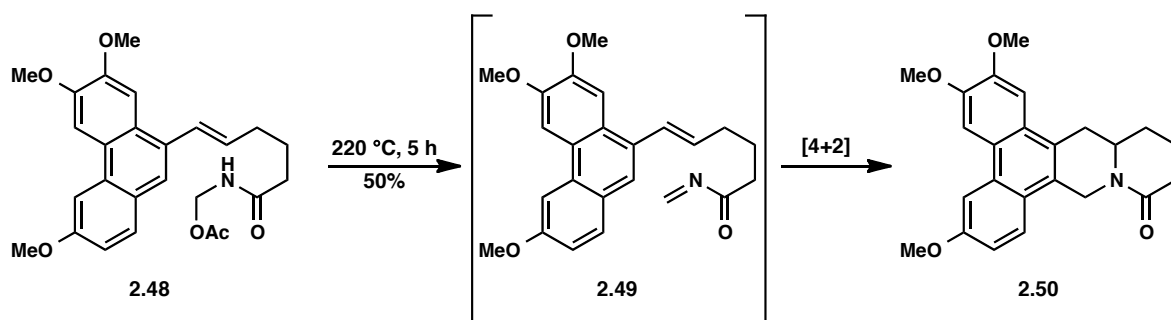
#### 2.1.6.2.4 Diels-Alder

The Diels-Alder reaction is a cycloaddition between a diene and a dienophile to form substituted 6-membered rings. The Diels-Alder is an electrocyclic reaction that involves the 4  $\pi$ -electrons of the diene and 2  $\pi$ -electrons of the dienophile. The driving force of the reaction is the formation of new  $\sigma$ -bonds, which are energetically more stable than the  $\pi$ -bonds. Fortunately, there are several reviews on this subject.<sup>281-286</sup>

A variant is the hetero-Diels-Alder reaction, in which either the diene or the dienophile contains a heteroatom, most often nitrogen or oxygen. This alternative constitutes a powerful method for the synthesis of six-membered ring heterocycles. That

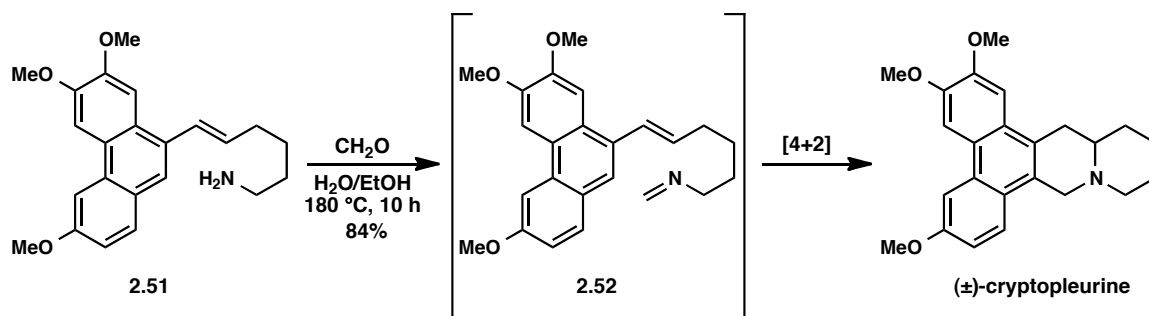
makes this approach particularly intriguing in the synthesis of phenanthropiperidines. Like the Pictet-Spengler, the Diels-Alder approach relies on a reactive imine. In the case shown in Scheme 2-10, the reactive imine **2.49** is formed by thermal decomposition of **2.48**.<sup>287</sup> Intermediate **2.49** undergoes a [4+2] cycloaddition to give quinolizidine **2.50**, which was then taken forward toward the synthesis of cryptopleurine.<sup>288</sup>

**Scheme 2-10.** Diels-Alder approach towards the synthesis of cryptopleurine



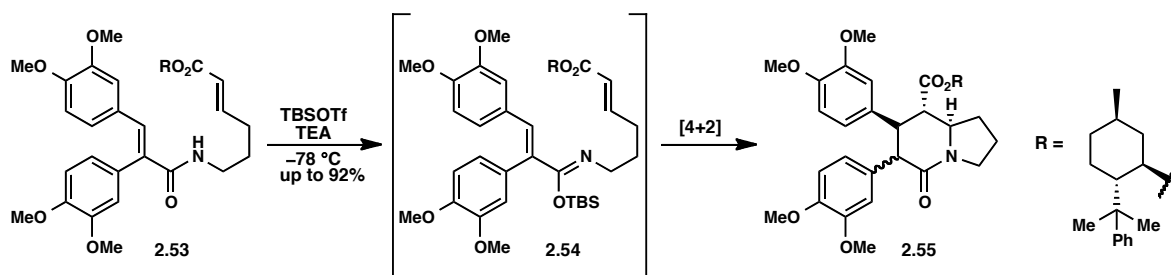
Another example of this approach, which on the face of it, looks more like a Pictet-Spengler reaction is shown in Scheme 2-11. The reactive imine **2.52** was generated by reacting amine **2.51** with formaldehyde at elevated temperature. The imine then undergoes a [4+2] cycloaddition to give (±)-cryptopleurine.<sup>289</sup> This approach was also used to synthesize (±)-lupine, (±)-epilupine, and (±)-julandine.<sup>289</sup>

**Scheme 2-11.** Diels-Alder approach for the synthesis of cryptopleurine



Given the success of the Diels-Alder approach, one question that needed to be addressed was whether enantioselective variants could be developed. In one approach chiral auxiliaries were employed. Using phenylmenthyl as the chiral auxiliary, stilbene **2.53** was treated with TBSOTf and TEA to form intermediate **2.54** which then underwent formal [4+2] cycloaddition to give indolizidine **2.55**.<sup>290</sup> Indolizidine **2.55** was then taken forward towards the synthesis of tylophorine. This approach forms four stereocenters in a single step, even though two of them are destroyed during the oxidation to form the phenanthrene system, and another one is removed by hydrolysis and decarboxylation. This was the first asymmetric synthesis of tylophorine.

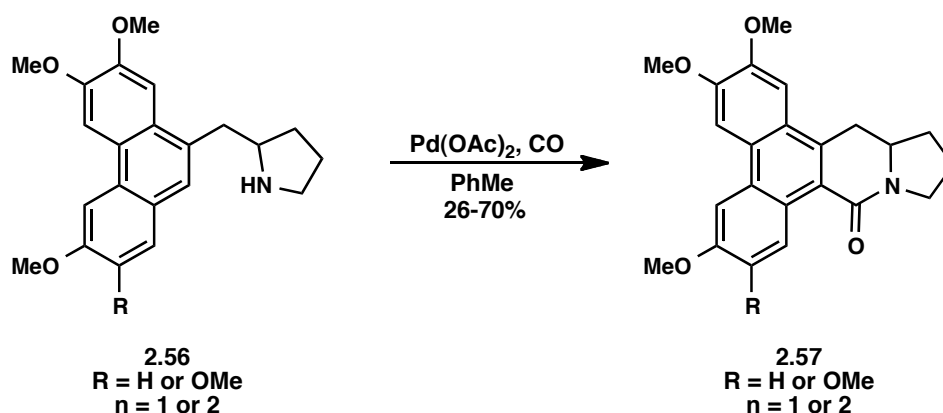
**Scheme 2-12.** Diels-Alder approach for the synthesis of tylophorine using a chiral auxiliary



### 2.1.6.2.5 Transition metal reactions

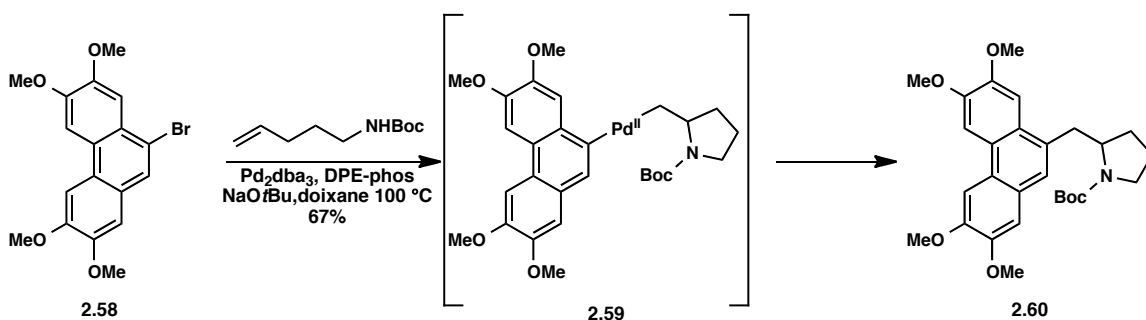
Transition metal chemistry has revolutionized the world of organic synthesis, the recognition of which led to the award of the 2010 Nobel Prize to Richard F. Heck, Ei-ichi Negishi, and Akira Suzuki for their work on palladium catalyzed cross couplings.<sup>291</sup> In the past few decades, late transition metals such as palladium, iron, copper, rhodium, and gold catalyzed reactions have played vital roles in the synthesis of complex molecules. The phenanthropiperidines are no different in this manner. Several groups have developed transition metal mediated methods to prepare the natural products. In formal syntheses of ( $\pm$ )-antofine, ( $\pm$ )-tylophorine, and ( $\pm$ )-cryptopleurine direct Pd-mediated carbonylation reactions yielded lactams of the natural products (Scheme 2-13).<sup>292</sup> While this direct method looks good on the surface, low yields were reported unless stoichiometric quantities of Pd were used.

**Scheme 2-13.** Direct Pd-mediated carbonylation



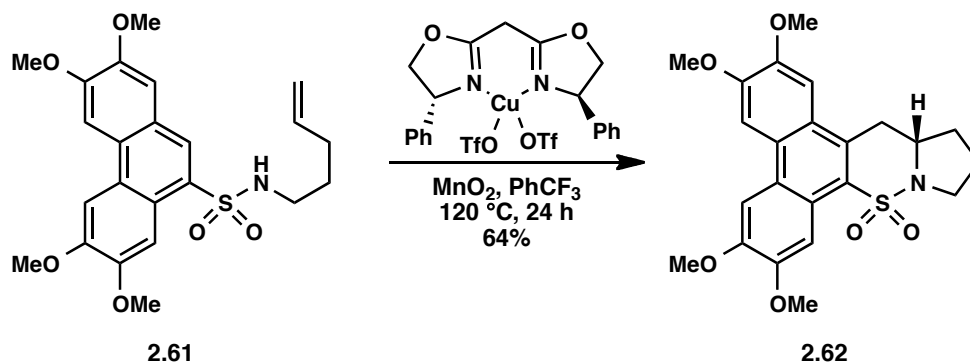
Another Pd-mediated method has been developed that uses the phenanthryl bromide **2.58** to yield the carboamination product **2.60** (Scheme 2-14).<sup>293</sup> In this scenario, the Pd oxidatively inserts in the aryl-halogen bond. The Pd species then coordinates to the alkene and the pendant carbamate, which then cyclizes onto the Pd-activated alkene to give pyrrolidine **2.59**. Reductive elimination gives the carboamination product **2.60**. This method was used to complete the total synthesis of tylophorine.<sup>293</sup>

**Scheme 2-14.** Pd-catalyzed carboamination



The last of the transition metal mediated reactions that will be discussed is the enantioselective Cu-catalyzed carboamination of sulfonamide **2.61** to give sultam **2.62** (Scheme 2-15).<sup>278</sup> Similar to the previous method, the carboamination provides direct access to a tylophorine precursor in relatively short order. Sultam **2.62** was then subjected to SO<sub>2</sub> elimination and a Pictet-Spengler reaction to give (*S*)-tylophorine in 81% enantiomeric excess.

**Scheme 2-15.** Enantioselective Cu-catalyzed carboamination



**2.1.6.2.6 N-alkylation reactions**

*N*-Alkylation reactions historically have been some of the most reliable reactions. The sheer number of available methods makes this reaction an obvious choice for synthetic chemists. The first method to use this approach towards the synthesis of the (*S*)-tylophorine was pioneered by Comins and coworkers and involves the synthesis of enaminones from substituted 4-methoxypyridinium **2.63** (Scheme 2-16).<sup>33</sup> This method has stood the test of time and been used in several natural product syntheses.<sup>6,8,294-296</sup> Once the enaminone carbamate **2.64** is deprotected, the resultant amine spontaneously cyclizes to the bicyclic enaminone **2.65**. The bicyclic enaminone is then taken forward in the synthesis of (*S*)-tylophorine.

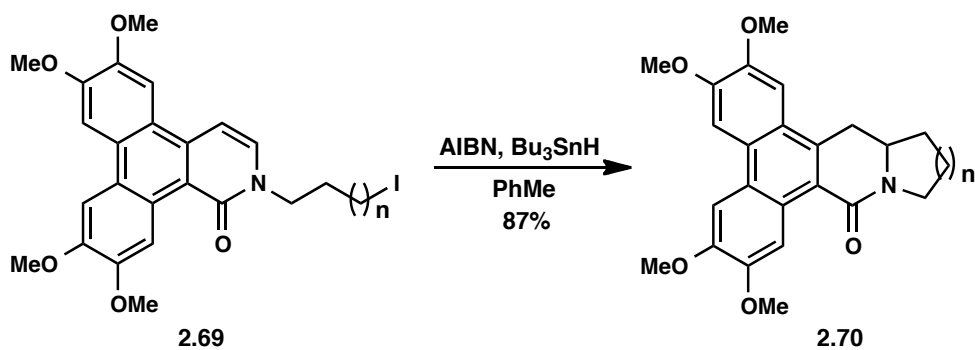




### 2.1.6.2.7 Radical cyclizations

Radical reactions play an important role in various aspects of chemistry, including organic chemistry and biochemistry. Free radicals are compounds with unpaired electrons derived from the homolytic cleavage of a bond. Heat, light, or an initiator forms the radical. This type of reaction has been successful in the synthesis of phenanthropiperidines.<sup>159,180</sup> In the case shown in Scheme 2-18, the alkyl iodides **2.69** were treated with the radical initiator AIBN to give the desired indolizidine and quinolizidine intermediates **2.70**. This method has been used to synthesize tylophorine, antofine, deoxypergularinine, and a few other analogs.<sup>159,180</sup>

**Scheme 2-18.** Free radical cyclization towards the synthesis of phenanthropiperidines

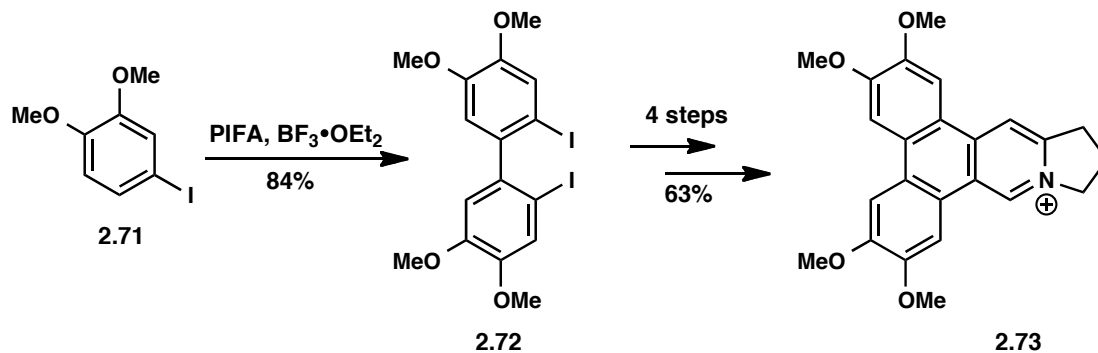


### 2.1.6.3 Synthesis of the phenanthrene system

There are relatively few methods available for the synthesis of the phenanthrene component of the phenanthropiperidines, and the point at which the phenanthrene synthesis is realized varies significantly between syntheses. There are several advantages to incorporating the phenanthrene earlier into the synthesis. The phenanthrene system is generally stable to most reaction conditions and usually a crystalline solid.

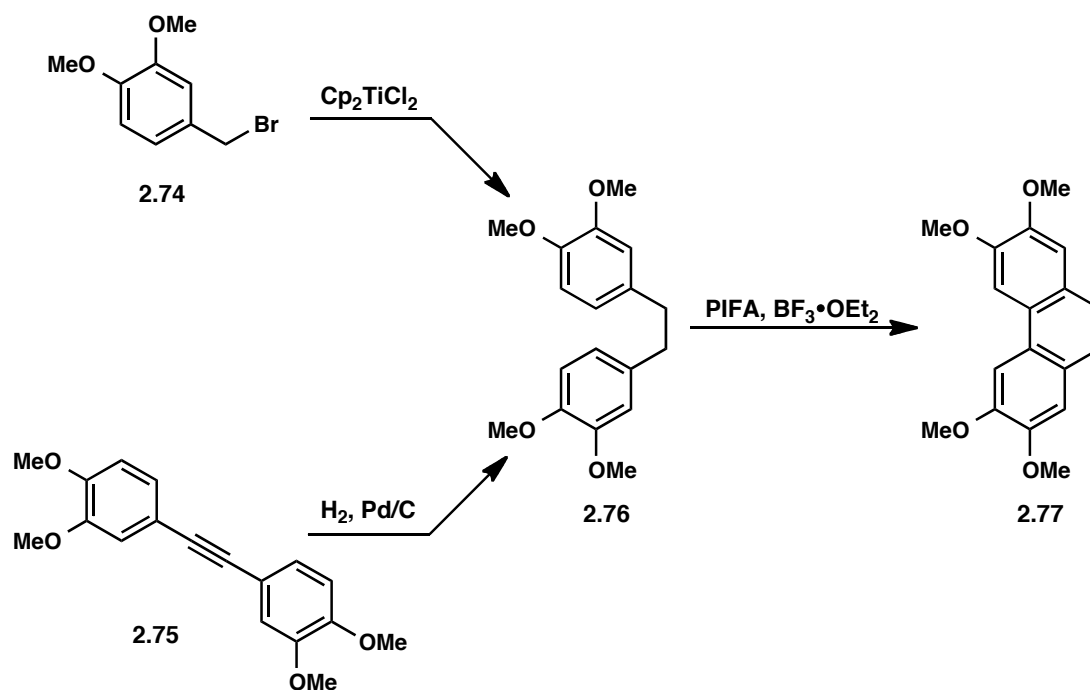
The symmetrical methoxy substitution pattern of tylophorine can be exploited to prepare the phenanthrene system through dimerization. Deiters and coworkers used this exact reasoning by dimerizing 4-iodoveratrole **2.71** to yield biaryl di-iodide **2.72** (Scheme 2-19).<sup>298</sup> Biaryl di-iodide **2.72** was then converted to a biaryl diyne in 3 steps, which set the stage for [2+2+2]-cyclotrimerization with a cyano mesylate to furnish dehydrotylophorine **2.73**. Dehydrotylophorine was reduced with NaBH<sub>4</sub> to yield tylophorine.

**Scheme 2-19.** Dimerization of 4-iodoveratrole to synthesize phenanthrene system



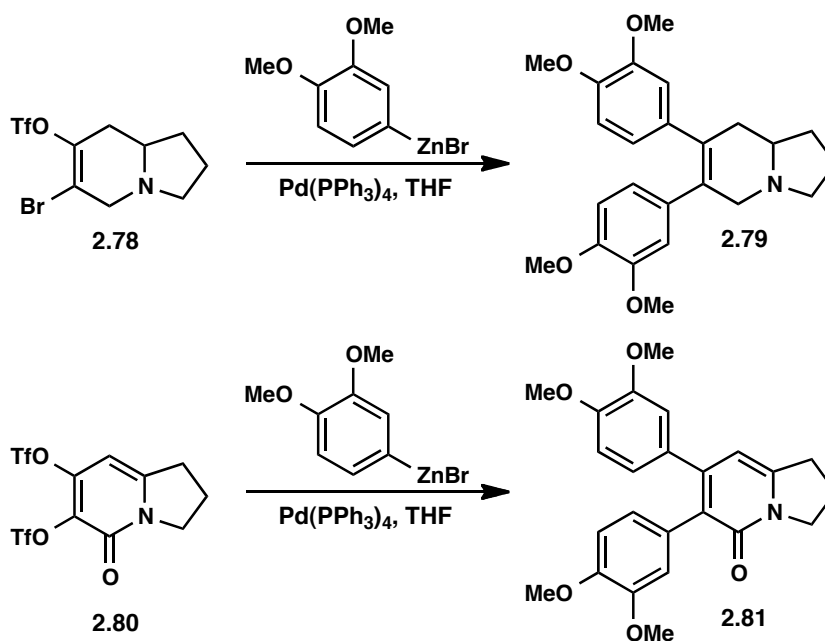
Another example of a dimerization approach is the radical induced dimerization reported by Chemler *et al.* (Scheme 2-20). The titanocene coordinates to the benzyl bromide **2.74** and homolytic cleavage forms the benzylic radical, which dimerizes to form the phenanthrene precursor **2.76**. A PIFA induced oxidative-coupling results in the phenanthrene **2.77**. Alternatively, the same phenanthrene can be synthesized from a very different route. The symmetrical acetylene **2.75** can be reduced using catalytic Pd/C under a hydrogen atmosphere gives the same phenanthrene precursor **2.76**, which is then transformed into the phenanthrene using the same conditions as previously discussed. The phenanthrene can be further functionalized to the bromo- or sulfonylphenanthrene using NBS or chlorosulfonic acid respectively.<sup>278,293</sup>

**Scheme 2-20.** Dimerization strategy towards the synthesis of the phenanthrene



The symmetrical phenanthrene has also been incorporated into later parts of the synthesis in a stepwise fashion. This work, shown elegantly by Comins<sup>32,33</sup> and Padwa,<sup>299</sup> uses Pd catalyzed bis-arylation reactions to install the requisite aryl rings (scheme 2-21) that have to undergo a subsequent oxidative phenanthrene forming reaction.

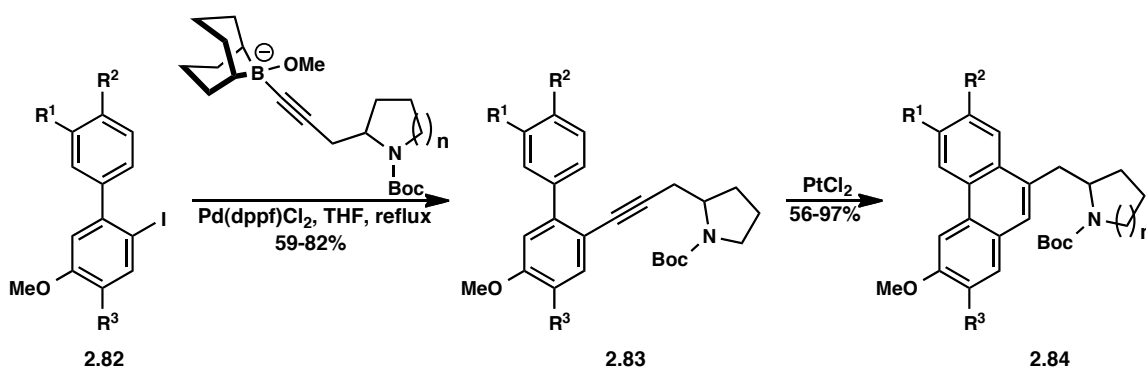
**Scheme 2-21.** Bisarylation strategy to synthesize phenanthrene precursors



The synthesis of the phenanthrene towards the end of the route has also been used for the synthesis of non-symmetrical phenanthrenes. For example, Fürstner *et al.* have reported the synthesis of the requisite biaryl iodides via Suzuki couplings (Scheme 2-

22.<sup>277</sup> The biaryl iodides **2.82** are coupled to the appropriate alkyne building block via a 9-MeO-9-BBN mediated cross coupling. Exposure of the alkyne **2.83** to PtCl<sub>2</sub> induced a cycloisomerization to form the phenanthrene system **2.84**. Boc-deprotection with concomitant Pictet-Spengler reaction formed the natural products. This route was used to synthesize (-)-tylophorine, (-)-antofine, (±)-cryptopleurine, and (-)-ficuseptine C.<sup>277</sup>

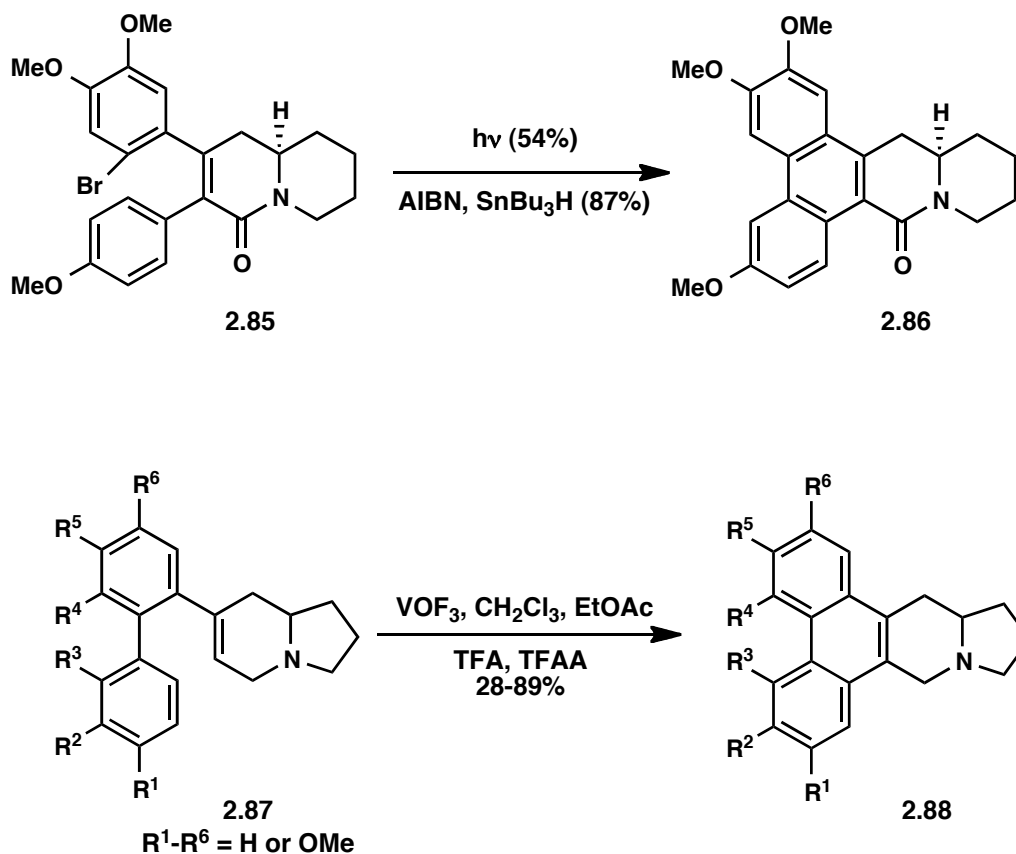
**Scheme 2-22.** Cycloisomerization strategy towards the synthesis of unsymmetrical phenanthrenes



Other methods have been developed towards the synthesis of unsymmetrical substituted phenanthrenes and are shown in Scheme 2-23. Kibayashi and co-workers used a radical reaction from julandine bromide **2.85** (*seco*-cryptopleurine) to close the phenanthrene system. In this case both light and the radical initiator AIBN with SnBu<sub>3</sub>H could invoke the formation of the phenanthrene **2.86**.<sup>256</sup> More recently Georg coworkers were able to induce the oxidative phenanthrene formation from an aryl-alkene precursor **2.87**.<sup>300</sup> This approach was used to synthesize (*R*)-tylophorine, (*R*)-tylocrebrine, (±)-

antofine, and other indolizidine analogs. There are several advantages to this approach. First, and foremost, is that this approach is convergent, so it can be amended to the phenanthrene substitution desired. Secondly, a systematically designed SAR can be designed around each part of the phenanthropiperidine without changing the synthetic route.

**Scheme 2-23.** Approaches towards the synthesis of unsymmetrical phenanthrenes



### **2.1.7 Summary of phenanthropiperidines**

The phenanthropiperidines have long been pursued as drug leads for their medicinal properties. Even though they have been pursued for nearly half a century, they have yet to make an impact clinically. Many members of this class show potent anti-proliferative and anti-inflammatory activity, on par with current therapeutics. It is likely that they operate under multiple mechanisms of action. Evidence has suggests that their anti-proliferative activity arises by inhibition of protein synthesis by binding to the 40S ribosomal subunit. Other evidence suggests that the phenanthropiperidines also disrupt the NF- $\kappa$ B signaling pathway and intercalate into DNA and RNA. However, the major hurdles to clinical success are the poor physiochemical properties and the neurological toxicity encountered in the brief clinical study. Thus, the discovery of new analogs that can mitigate the off target interactions as well as the improvement of the physiochemical properties will decide the fate of this class of alkaloids.

## **2.2 Synthesis and biological evaluation of boehmeriasin A, tylocrebrine, and related alkaloids**

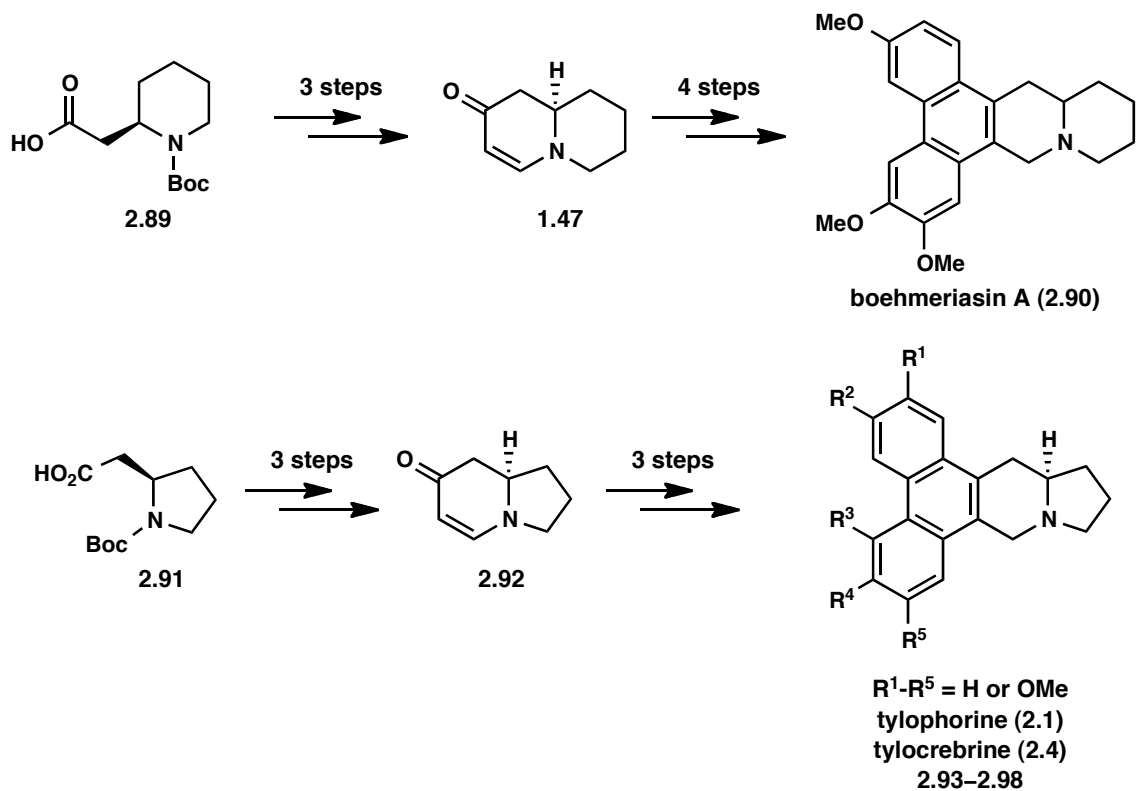
With the phenanthropiperidines being intensely pursued as drug leads, our group previously prepared several phenanthropiperidines in order to further assess their therapeutic potential. As discussed earlier in this chapter, the major concern arises from the failed clinical trial of tylocrebrine, which possessed undesired neurological side



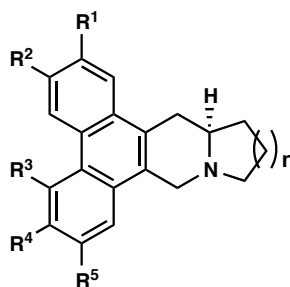
effects. The molecular basis for this off-target interaction has only recently been investigated. Hence, in order to design analogs, we must study the CNS activity of phenanthropiperidines and related analogs to find the relevant CNS target(s). In another approach, analogues could be designed that do not cross the BBB.

The first step in that direction was to complete the synthesis of the phenanthropiperidines of interest and confirm their biological activity. Toward this end, previous members of our group synthesized boehmeriasin A, tylocrebrine, and tylocrebrine related alkaloids (Scheme 2-24) from key intermediates **1.47** and **2.92**.<sup>300,301</sup>

**Scheme 2-24.** Synthesis of boehmeriasin A, tylocrebrine, and tylocrebrine related alkaloids



The synthetic analogs were then tested for their cytotoxicity against several cancer cell lines (Table 2-11). Boehmeriasin A showed significant cytotoxicity against all of the cancer cell lines tested (Table 2-11, entry 1). Most importantly, it possesses potency against multi-drug resistant cell lines. Tylophorine and tylocrebrine also showed significant activity against the cell lines (Table 2-11, entries 2 and 3). Tylophorine displayed decrease activity against multi-drug resistant cell lines, when compared to boehmeriasin A and tylocrebrine. The synthetic analogs were consistently active against the cell lines except for the multi-drug resistant cell line, which was more sensitive toward modification. The retention of activity across the series suggests that the phenanthropiperidines are well suited for further drug development.

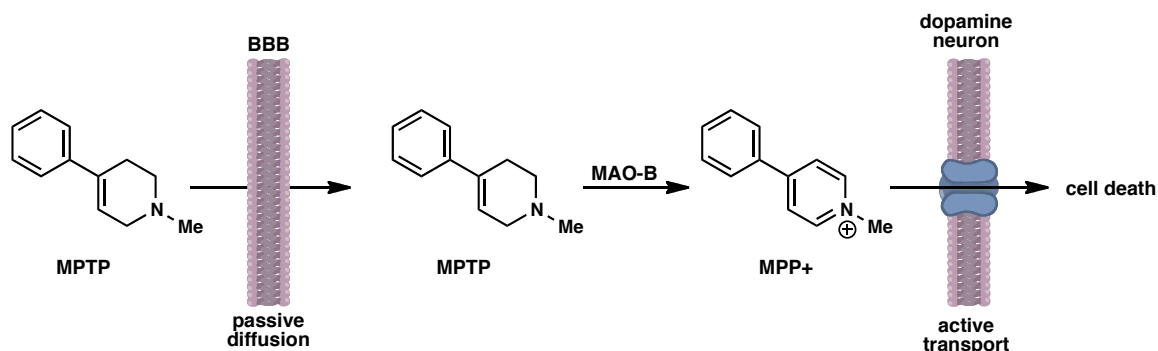
**Table 2-11.** Cytotoxic activity of phenanthropiperidines

| entry | compound name/number  | R <sup>1</sup> | R <sup>2</sup> | R <sup>3</sup> | R <sup>4</sup> | R <sup>5</sup> | n | IC <sub>50</sub> (nM) |       |             |
|-------|-----------------------|----------------|----------------|----------------|----------------|----------------|---|-----------------------|-------|-------------|
|       |                       |                |                |                |                |                |   | COLO-205              | MCF-7 | NCI-ADR-RES |
| 1     | boehmeriasin A (2.90) | H              | OMe            | H              | OMe            | OMe            | 2 | 4..2                  | 43    | 37          |
| 2     | tylophorine (2.1)     | OMe            | OMe            | H              | OMe            | OMe            | 1 | 17                    | 32    | 160         |
| 3     | tylocrebrine (2.4)    | OMe            | OMe            | OMe            | OMe            | H              | 1 | 1.3                   | 15    | 26          |
| 4     | 2.93                  | OMe            | OMe            | OMe            | H              | H              | 1 | 9.5                   | 22    | 16          |
| 5     | 2.94                  | OMe            | OMe            | H              | OMe            | H              | 1 | 2.8                   | 21    | 23          |
| 6     | 2.95                  | OMe            | OMe            | H              | H              | OMe            | 1 | 270                   | 530   | 1300        |
| 7     | 2.96                  | H              | OMe            | H              | OMe            | OMe            | 1 | 8.5                   | 5.8   | 200         |
| 8     | 2.97                  | H              | H              | H              | OMe            | OMe            | 1 | 48                    | 25    | 180         |
| 9     | 2.98                  | OMe            | OMe            | H              | H              | H              | 1 | 29                    | 21    | 180         |

<sup>a</sup>COLO-205 ) human colorectal adenocarcinoma; MCF-7 ) human breast carcinoma; NCI-ADR-RES ) drug-resistant human ovarian adenocarcinoma.

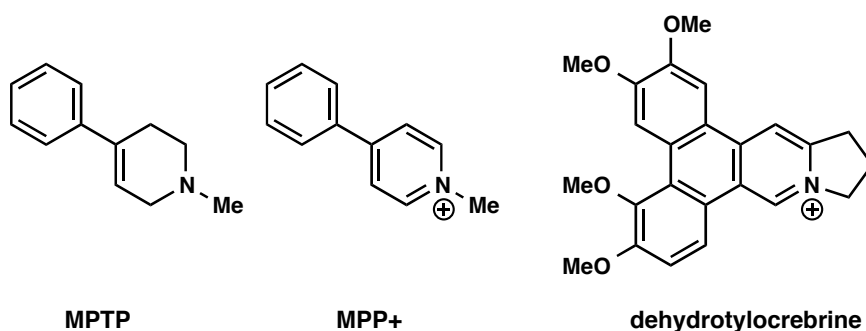
With the synthetic analogs on hand, an investigation was performed to find the cause of the neurological side effects. The absence of clinical data made this search difficult. As reported by Suffness,<sup>104</sup> tylocrebrine interferes with voluntary movement at some level. Thus, two potential mechanisms of action were explored based on tylocrebrine's structural similarity to bioactive molecules known to alter motor function, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and dopamine.<sup>302</sup>

Research has shown that MPTP undergoes a sequence of events that lead to dopamine neuronal cell death. Because MPTP is lipophilic, it passively diffuses across the BBB to enter the CNS. Once in the CNS, MPTP is oxidized by monoamine oxidase-



**Figure 2-11.** Mechanism of MPTP neurotoxicity.

B (MAO-B) to 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>), the active toxin. MPP<sup>+</sup> is actively transported into dopamine neurons, eventually shutting down cellular metabolism leading to cell death (Figure 2-11). The major concern is that the structure of MPTP is embedded within tylocrebrine (Figure 2-12). This hypothesis was examined using the MAO-Amplex® Red assay, which measures H<sub>2</sub>O<sub>2</sub> as a byproduct of the oxidation.<sup>302</sup> The results showed that in concentrations up to 100 μM tylocrebrine or other phenanthropiperidines were not substrates for monoamine oxidase A or B (data not shown). This demonstrated that oxidation via this mechanism is unlikely, but does not rule out oxidation by other metabolic enzymes or non-enzymatic oxidation.<sup>303</sup>



**Figure 2-12.** Structural similarities between MPTP, MPP+, and tylocrebrine.

As stated earlier, another concern was that tylocrebrine was blocking neurotransmission by mimicking biogenic amines, such as dopamine. Again, the dopamine structure is embedded within the tylocrebrine structure. Dopamine, a key-signaling molecule, plays a role in motor control, cognition, emotion, and many other functions.<sup>304</sup> An additional piece of evidence is that abnormalities of the dopaminergic system in the substantia nigra have been implicated in Parkinson's disease, characterized by loss of voluntary movement/motor control. Even though dopamine may be a likely target, there is a considerable amount of overlap between the dopamine receptor ligands and other biogenic amine receptors, such as adrenergic, serotonergic, muscarinic, histaminergic, and opioid receptors. Thus, the collection of synthesized phenanthropiperidines was submitted to the National Institutes of Mental Health-Psychoactive Drug Screening Program for a comprehensive CNS-target screen (data not shown). The primary screen identified compounds that had significant binding (defined as greater than 50% displacement of a radioligand) to receptors at 10  $\mu$ M.<sup>305</sup> This was more fruitful than the previous hypothesis. Tylocrebrine was found to have significant binding at numerous CNS receptors across several types (serotonergic, adrenergic, dopaminergic, muscarinic, and histaminergic). Focusing on the dopaminergic receptor affinity, tylocrebrine had high-moderate affinity for the D<sub>1</sub> and D<sub>5</sub> receptors and moderate-low affinity for D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, and receptors. The D<sub>1</sub> is the most abundant receptor in the CNS<sup>303</sup> as well as expressed outside the CNS,<sup>306,307</sup> thus, modulating the D<sub>1</sub> receptor could have wide ranging physiological consequences. Tylophorine, on the other

hand, was relatively clean across the receptor screen having only moderate affinity for a few adrenergic receptors.

These neurological studies have answered some questions, but left several unanswered. The first being that oxidation via MAO-B is likely not the cause of the neurotoxicity, but does not rule out other enzymatic or non-enzymatic oxidation of the phenanthropiperidines to form MPTP-like products. The NIMH-PDSP screen revealed several CNS receptor interactions. Tylocrebrine had high-moderate binding to dopamine D<sub>1</sub> and D<sub>5</sub> receptors. A secondary screen revealed that tylocrebrine had only moderate affinity (K<sub>D</sub> 448 nM) for the D<sub>1</sub> receptor, raising more questions than answers.<sup>305</sup> While this information is useful, a functional assay would elucidate tylocrebrine's efficacy upon binding to the receptor in question. Thus, the mechanism of tylocrebrine-induced neurotoxicity is still not understood.

### **2.3 Simplified tylophorine analogs**

One strategy for precluding the neurological toxicity is the design of non-BBB penetrating analogs. Since this approach does not require a mechanistic understanding, we decided to explore this idea. This approach does come with its limitations; however, tumors within the CNS would not be treatable.

Designing phenanthropiperidines that do not cross the BBB should be more straightforward than the design of analogs that do not interact with specific molecular targets. As discussed earlier in this chapter, CNS exposure has been linked to physiochemical properties that can be adjusted to help or hinder BBB-permeation.<sup>251</sup>

When these properties are taken into account, it is no surprise that tylocrebrine enters the CNS (Table 2-10). Toward this end, the design of such analogs should be directed toward increasing the polarity of the phenanthropiperidine without affecting their cytotoxicity. Furthermore, imparting physiochemical properties that preclude BBB-permeation will also improve water solubility. From what is already known about the SAR there are several promising sites for introducing polar functional groups. The C3, C6, and C14 sites are somewhat insensitive toward modification and could be used to install polarizing functional groups. The E ring is virtually unexplored, besides being sensitive toward heteroatom incorporation.<sup>188</sup> Modifications of the E-ring will provide additional knowledge of SARs and if those modifications are tolerated they might provide the opportunity to add polar functional groups, thereby increasing solubility and possibly precluding BBB-permeation.

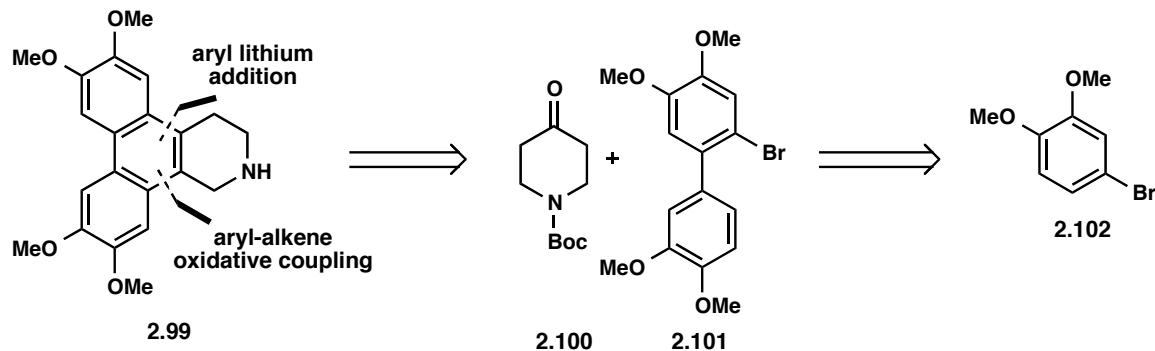
Herein, we disclose the synthesis and biological evaluation of a library of simplified tylophorine analogs. Tylophorine was used as the as a representative, because it is one of the most well studied members of its class, and due to its relatively clean CNS target screen. Simplified analogs lacking the E-ring would, consequently, eliminate the sole stereogenic center and dramatically simplify the synthesis.

### **2.3.1 Synthesis of simplified tylophorine analogs**

In order to obtain sufficient quantities of the library precursor, we first devised a concise synthetic plan to prepare **2.99**, which lacks the E-ring (Scheme 2-25). The nucleophilic secondary amine can then be further modified for SAR studies. Piperidine

**2.99** can be prepared from commercially available *N*-Boc-piperidone **2.100** and biaryl **2.101**. Biaryl **2.101** can be obtained from 4-bromoveratrole **2.102**.

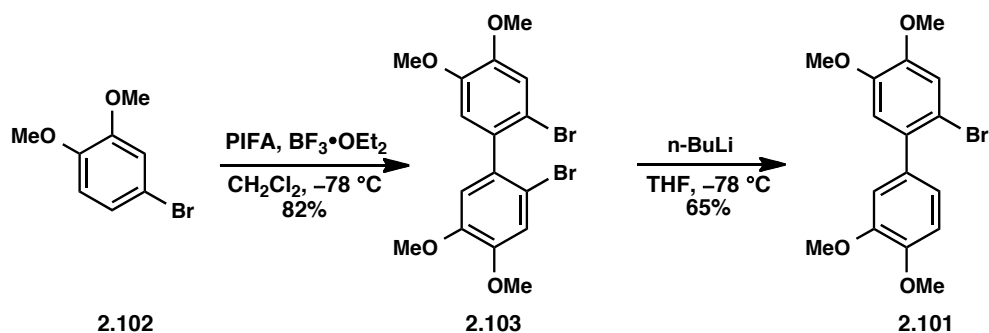
**Scheme 2-25.** Retrosynthetic analysis



We next set out to synthesize ample quantities of piperidine **2.99** for the synthesis of a library of compounds (Scheme 2-26). 4-Bromoveratrole (**2.102**) was dimerized using a PIFA-mediated oxidative coupling reaction to give biaryl dibromide **2.103**.<sup>308</sup> The resultant dibromoveratrole dimer was desymmetrized through a lithium-halogen exchange using a single equivalent of *n*-butyllithium to provide biaryl monobromide **2.101**. Notably, these two steps could be carried out on a large scale (>10 g) without relying on chromatography for purification. Following another lithium halogen exchange of biaryl monobromide **2.101** (Scheme 2-27), the biaryl lithium was added to *N*-Boc-

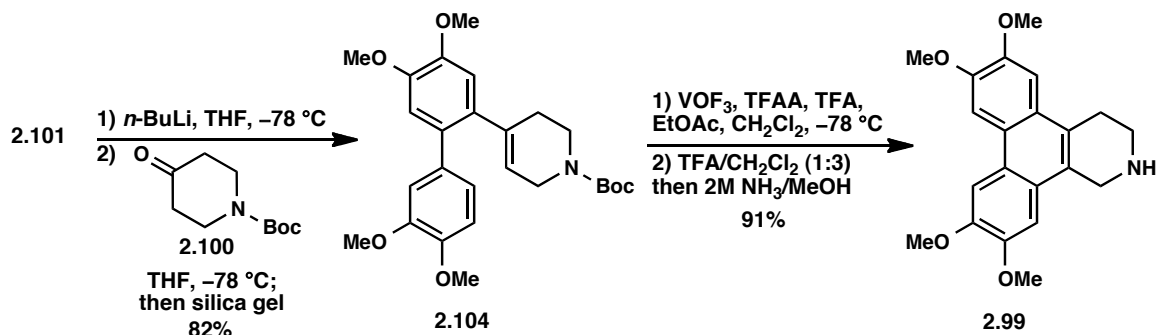


**Scheme 2-26.** Synthesis of biaryl monobromide



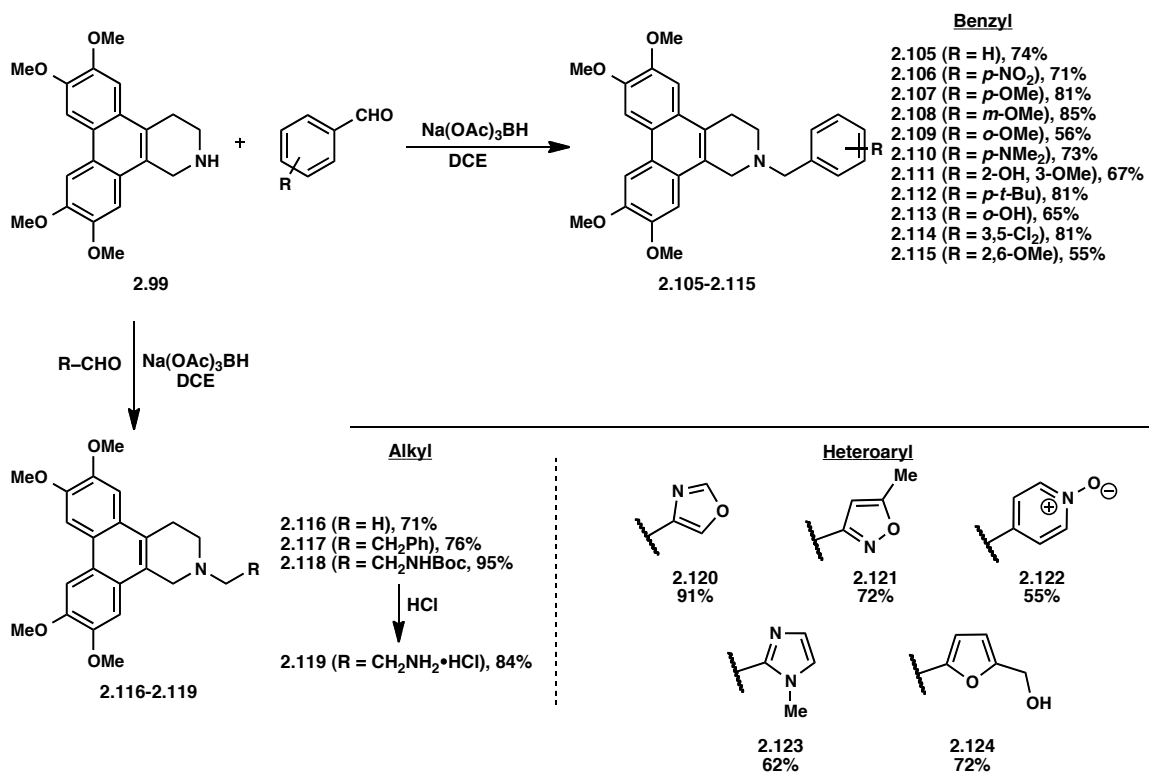
piperidone **2.100**, which upon exposure to silica gel spontaneously dehydrated to afford tetrahydropyridine **2.104**. Using our recently developed aryl-alkene oxidative coupling,<sup>300</sup> the phenanthrene system was constructed in excellent yields, providing protected piperidine **2.104**. Subsequent deprotection of the Boc group with TFA afforded the desired phenanthropiperidine **2.99**. This sequence provided gram quantities of the key intermediate, phenanthropiperidine **2.99**, in five steps and 61% overall yield

**Scheme 2-27.** Synthesis of phenanthropiperidine core



Previous SAR studies have shown that amide analogs of the phenanthropiperidines are substantially less active than the naturally occurring amines.<sup>180,189</sup> As such, we chose to prepare a library of *N*-alkylated phenanthropiperidines from phenanthropiperidine **2.99**. We decided that a reductive amination reaction would be most practical based on the commercial availability of a diverse collection of aldehydes and its amenability toward parallel synthesis. One point that should be noted is that phenanthropiperidine **2.99** is poorly soluble in most organic solvents, limiting the chemistry that can be used to derivatize this intermediate. Fortunately, halogenated solvents such as DCE and CH<sub>2</sub>Cl<sub>2</sub> could sufficiently solubilize the amine, albeit at low concentrations (0.02 M), to enable efficient *N*-alkylations with a diverse collection of commercially available aldehydes. The collection of aldehydes varied from aryl, heteroaryl, to alkyl aldehydes. In general, the reactions proceeded in excellent yield (55-95%) providing the desired benzyl- (**2.105-2.115**), heteroaryl- (**2.116-2.121**), and alkyl- (**2.122-2.125**) derivatives of tylophorine.

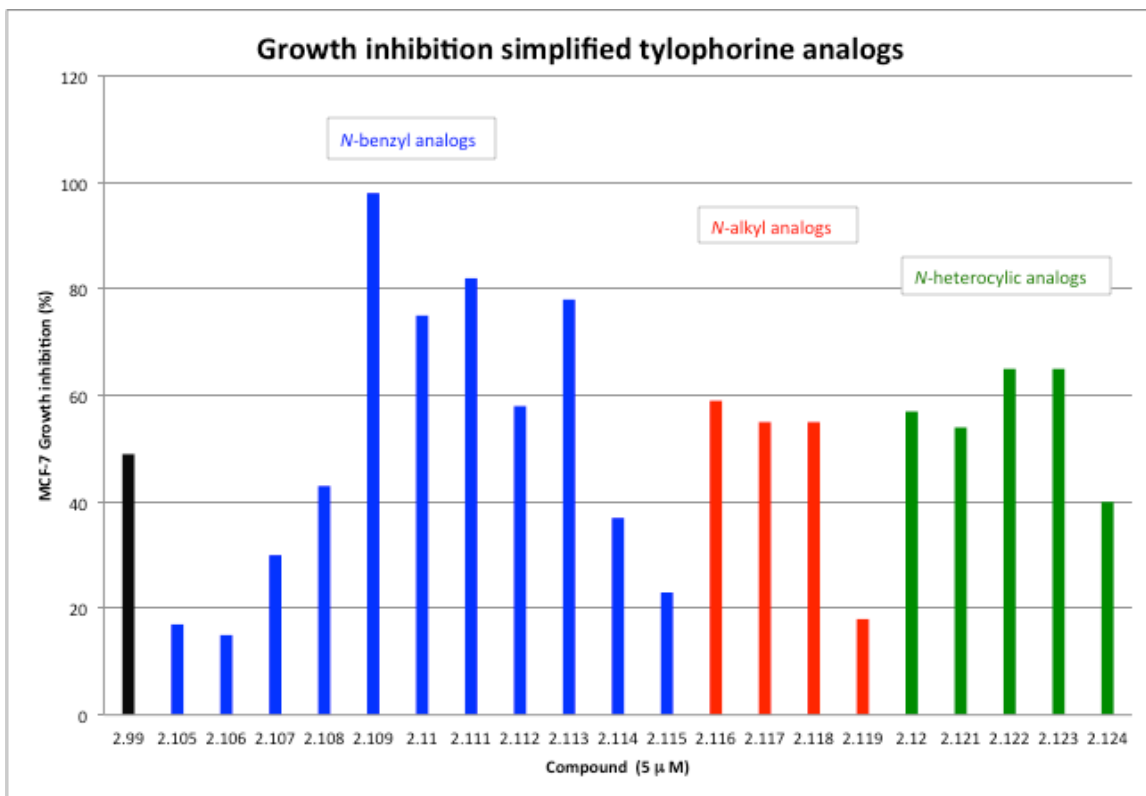
**Scheme 2-28.** Synthesis of *N*-substituted phenanthropiperidine library derived from **2.99**



**2.3.2 Biological evaluation of simplified tylophorine analogs**

The anti-proliferative activity of the phenanthropiperidines has been investigated in many cell lines.<sup>105</sup> The most well studied members of this class show little discrimination between the cell lines and even retain their activity in those resistant to the most commonly used anti-cancer drugs. As mentioned previously, the necessity of the E-ring is unexplored. In order to assess the potential of these compounds for further development,

we first tested the anti-proliferative activity of each compound at 5  $\mu\text{M}$  in MCF-7 (human breast carcinoma) cells (Figure 2-13).



**Figure 2-13.** Anti-proliferative activity of *N*-substituted phenanthropiperidines at 5  $\mu\text{M}$  in MCF-7 cells.

We found that many of the library members showed greater than 50% inhibition of cell growth at the 5  $\mu\text{M}$  concentration. Most compounds, however, were significantly less active than tylophorine. The unsubstituted phenanthropiperidine core **2.99** and the *N*-methyl analog **2.116**, the simplest library members, inhibited cell growth by 50-60% suggesting that the intact quinolizidine or indolizidine ring found in the natural products contribute significantly to their activity. Benzylation of the nitrogen resulted in a near

complete loss of activity. With that being said, a clear phenyl-substituent effect was observed for benzyl analogs. Para-substituted benzyl groups **2.106** and **2.107** displayed low activity, whereas ortho-substituted benzyl analogs **2.109**, **2.111**, and **2.113** were the most potent compounds of the library. The 2-methoxybenzyl analog **2.109** was particularly active showing a growth inhibition of ~90%. However, the addition of another ortho-methoxy substituent resulted in a reduction of activity (**2.115**). *N*-Alkyl substituted analogs **2.116-2.119** were equipotent to the phenanthropiperidine core **2.99**. Boc-deprotection of **2.118** significantly reduced its antiproliferative activity (**2.119**). Several heterocyclic analogs were also prepared with the intent that this modification would improve solubility, and possibly activity. Unfortunately, only a slight improvement of activity was observed for the two heterocyclic members, isooxazole **2.121** and furan **2.124**.

After obtaining these preliminary results, we analyzed selected compounds in more detail (Table 2-12). These compounds were assayed to determine their GI<sub>50</sub> values first against MCF-7 cells. The phenanthropiperidine core **2.99** had a GI<sub>50</sub> of 14.7 μM, suggesting that the E-ring plays role in the activity, thereby corroborating our previous screen. The ortho-methoxy compound turned out to be the most active again one with a GI<sub>50</sub> of 600 nM. The other compound that should be pointed out is **2.111** with a GI<sub>50</sub> of 2.1 μM was the second most active derivative. The alkyl and heteroaromatic compounds chosen for further evaluation mirrored the screen at 5 μM.

**Table 2-12.** GI<sub>50</sub> values of selected *N*-substituted phenanthropiperidines in MCF-7 cells

| GI <sub>50</sub> (μM) |            | GI <sub>50</sub> (μM) |            |
|-----------------------|------------|-----------------------|------------|
| compound              | MCF-7      | compound              | MCF-7      |
| <b>2.99</b>           | <b>15</b>  | <b>2.115</b>          | <b>1.0</b> |
| <b>2.109</b>          | <b>0.6</b> | <b>2.119</b>          | <b>33</b>  |
| <b>2.111</b>          | <b>2.1</b> | <b>2.121</b>          | <b>11</b>  |
| <b>2.113</b>          | <b>4.3</b> | <b>2.124</b>          | <b>5.9</b> |

MCF-7 = human breast carcinoma

One notable characteristic of the phenanthropiperidines is that they retain their activity across multiple cell lines. To see if that trend held true for the simplified tylophorine analogs, compounds **2.109** and **2.111** were selected for evaluation in other cell lines (Table 2-13). We found that the simplified tylophorine analogs retained their activity against DU-145 and A549 cells. The ortho-methoxy analog **2.109** was again the most active with GI<sub>50</sub> values of 1.6 and 1.6 μM respectively. The 2-hydroxy-3-methoxy analog **2.111** had a GI<sub>50</sub> of 2.1 μM in both cell lines. Motivated by previous reports that tylophorine inhibits NF-κB-mediated transcription,<sup>158</sup> we examined the effect of compounds **2.109** and **2.111** on NF-κB-mediated activity using A549 cells stably expressing the luciferase reporter gene downstream of NF-κB. Consistent with previous findings, tylophorine potently inhibited NF-κB activity with an IC<sub>50</sub> of 30 nM. On the other hand, analogs **2.109** and **2.111** only inhibited the pathway at concentrations greater than 10 μM. Considering the structurally sensitive nature of the mechanism of

action,<sup>173,188</sup> this data suggests that the simplified tylophorine analogs have a different mechanism of action than tylophorine.

**Table 2-13.** Antiproliferative and NF-κB activity of **2.109** and **2.111**

| compound        | GI <sub>50</sub> (μM) <sup>a</sup> |       | NF-κB inhibition <sup>b</sup><br>IC <sub>50</sub> (μM) |
|-----------------|------------------------------------|-------|--|
|                 | DU-145                             | A549  |  |
| (±)-tylophorine | 0.071                              | 0.045 | 0.03   |
| (S)-tylophorine | 0.1                                | 0.01  | –  |
| <b>2.109</b>    | 1.5                                | 1.6   | 15   |
| <b>2.111</b>    | 2.1                                | 2.1   | 19   |

<sup>a</sup> DU-145 = human prostate carcinoma; A549/NF-κB-luc = human lung carcinoma. <sup>b</sup> NF-κB inhibition was measured by monitoring the luciferase activity from A549/NF-κB-luc cell upon treatment with the indicated compound.

### 2.3.3 Summary of simplified tylophorine analogs

A library of simplified tylophorine analogs was prepared to explore the effects of *N*-substitution at the phenanthropiperidine core. The synthetic route developed enabled a highly efficient synthesis of the phenanthropiperidine scaffold from which each library member could be prepared through a reductive amination reaction. Despite having respectable antiproliferative activity, in general, library members had reduced activity when compared to tylophorine. A significant improvement in activity was observed in ortho-substituted benzyl analogs, the best of which, **2.109**, displayed a GI<sub>50</sub> value of 600 nM against MCF-7 cell proliferation. The lack of NF-κB activity suggests that these

structural analogs exert their antiproliferative activity through a mechanism of action that is different from that of tylophorine.

Given the synthetic accessibility of these analogs and the suitability of this route for library development, this work opens up new avenues to explore the phenanthropiperidine core. More importantly, this work sheds light on the necessity of the E-ring for the phenanthropiperidines potent antiproliferative activity for scaffold **2.99**.

## 2.4 Polar phenanthropiperidines

As mentioned earlier in this chapter, the design of polar analogs could mitigate the nervous system penetration.<sup>104</sup> In 2000, it was noted that the C14 hydroxy derivative of antofine *N*-oxide inhibited cell growth of KB-V1 and KB-3-1 cells with IC<sub>50</sub>'s of 160 and 100 nM respectively.<sup>125</sup> Gao and coworkers showed that DCB-3503 (Figure 2-5, **2.15**) inhibited cell growth of KB and HepG2 cells at 28 and 35 nM respectively.<sup>158</sup> Along with the excellent *in vitro* activity, they also noted superior *in vivo* activity. It was discovered that DCB-3503 inhibited the NF- $\kappa$ B signaling pathway through the suppression of IKK.<sup>163</sup> The C14-hydroxyantofine and the C15-hydroxycryptopleurine (see Figure 2-2 for indolizidine and quinolizidine numbering) have also been shown to potently inhibit *in vitro* cell growth in the low nanomolar range.<sup>229,309</sup> These examples demonstrate that certain polar phenanthropiperidines retain the potent antiproliferative activity of phenanthropiperidines. In the case of DCB-3503, superior *in vivo* activity was also observed. It can be speculated that this is due to the small increase in solubility that the hydroxyl group would provide. This result provided precedence for us to incorporate a

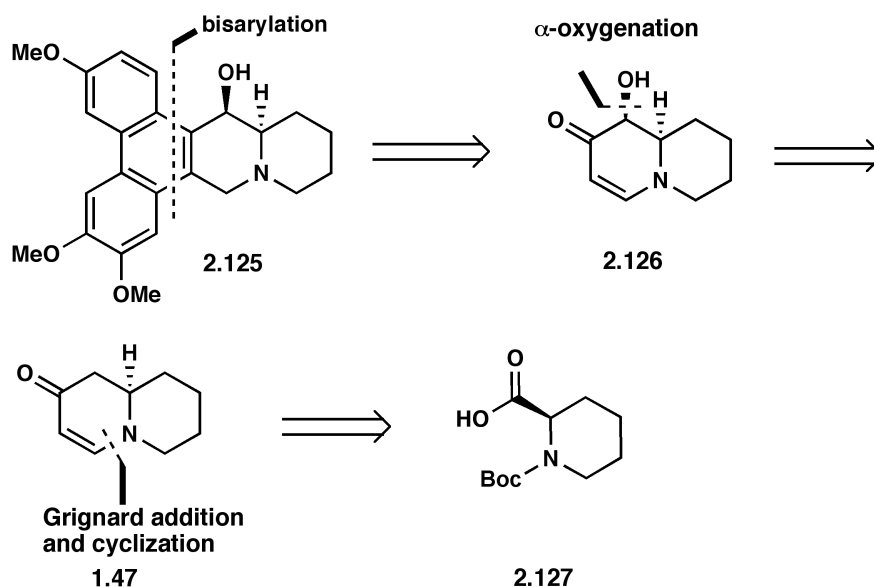


hydroxy group at the C15-position of boehmeriasin A. We not only wanted to incorporate the hydroxy group to increase the polarity of boehmeriasin A, but could use that group as a functional handle to incorporate other water solubilizing moieties.

#### 2.4.1 Synthesis of (*R*)-15-Hydroxyboehmeriasin A

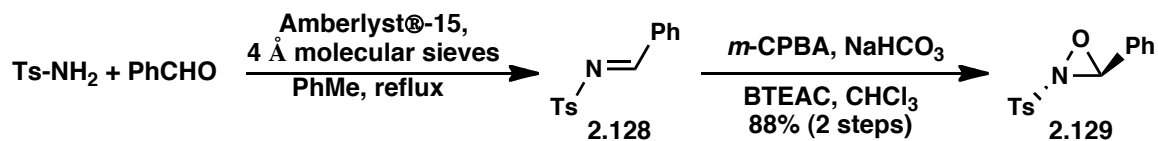
Retrosynthetically (Scheme 2-29) we envisioned that 15-hydroxyboehmeriasin A **2.125** could arise from  $\alpha$ -OH-6,6-enaminone **2.126**, which could be derived from enaminone **1.47**, and can be prepared from pipercolic acid **2.127**. In order to install the  $\alpha$ -hydroxy group, we planned to use *N*-tosyloxaziridine as an electrophilic oxygen transfer reagent.<sup>311</sup>

**Scheme 2-29.** Retrosynthetic analysis of 15-Hydroxyboehmeriasin A



We first had to synthesize the oxaziridine **2.129** (Scheme 2-30). We used an Organic Synthesis preparation that allowed us to prepare >50g of the oxaziridine **2.129** in excellent yields and purity.<sup>311</sup> *p*-Toluenesulfonamide was condensed with benzaldehyde in the presence of an acid catalyst (Amberlyst®-15) at refluxing temperatures to provide imine **2.128**. Imine **2.128** was oxidized using different procedures. First, we used oxone because this reagent was reported to be a more efficient oxidant providing oxaziridines in 40-50% yield.<sup>311</sup> However we found that *m*-CPBA was a superior and provided the oxaziridine **2.129** in excellent yields (typically >90%) in just over 1 h after a single recrystallization.<sup>311</sup>

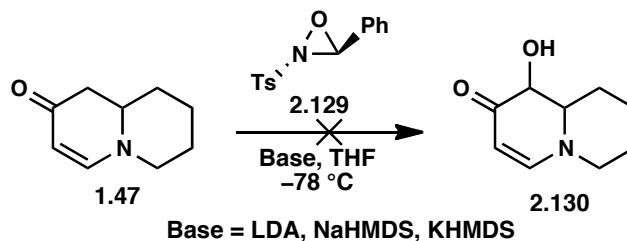
**Scheme 2-30.** Synthesis of *N*-tosyloxaziridine **2.129**



With the oxaziridine on hand, we set out to install the oxygen functionality at the  $\alpha$ -position of the 6,6-enaminone **1.47** (Scheme 2-31). In our first attempt we used LDA as a base at  $-78$  °C, because of previous success in our group using it to form the enolate and quenching the anion with Manders' reagent to form the methyl ester at the  $\alpha$  position. Unfortunately, enolate formation with LDA failed to provide any product, as only starting materials were recovered (Scheme 2-31). Raising the temperature slowly to 0 °C led only

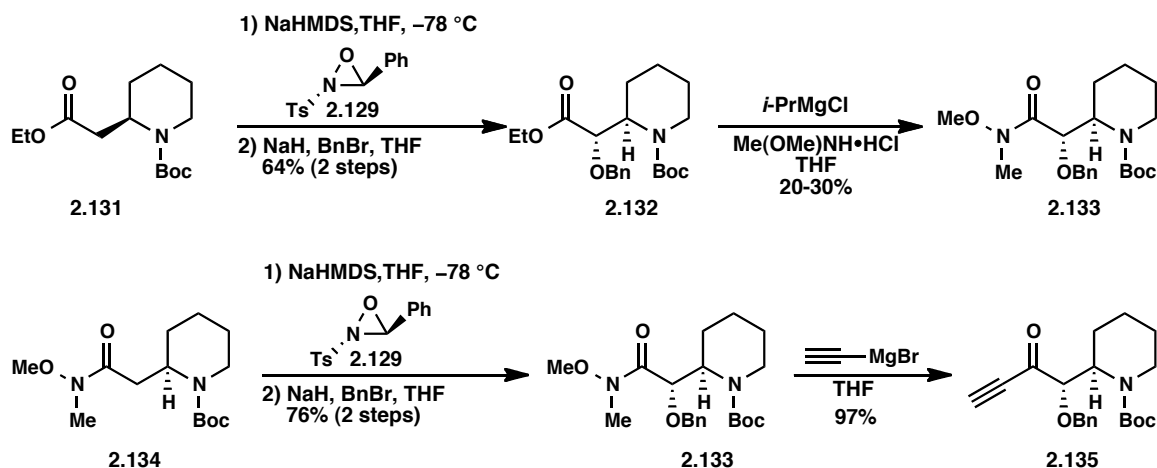
to decomposition. The same results were obtained with NaHMDS or KHMDS as bases. These negative results led us to pursue a different route.

**Scheme 2-31.** Initial attempts at installing the hydroxy group



We next tried to install the hydroxy group to the  $\beta$ -amino ester **2.131**. This approach was successful in providing the  $\alpha$ -hydroxy- $\beta$ -amino ester **2.132** in 78% yield as a single diastereomer. However, after protection of the alcohol as its benzyl ether, the next amidation step proceeded in very poor yields (ca ~20-30%). Since it is known that Weinreb amides can undergo  $\alpha$ -oxygenation using basic conditions and oxaziridines,<sup>312</sup> we explored this option using  $\beta$ -amino amide **2.134**.  $\beta$ -Amino-amide **2.134** underwent  $\alpha$ -oxygenation readily with better yields than the  $\beta$ -amino ester **2.131** (85% vs 78%), again as a single diastereomer (Scheme 2-33). After protection, the  $\alpha$ -benzyl ether did not inhibit the formation of ynone **2.140**.

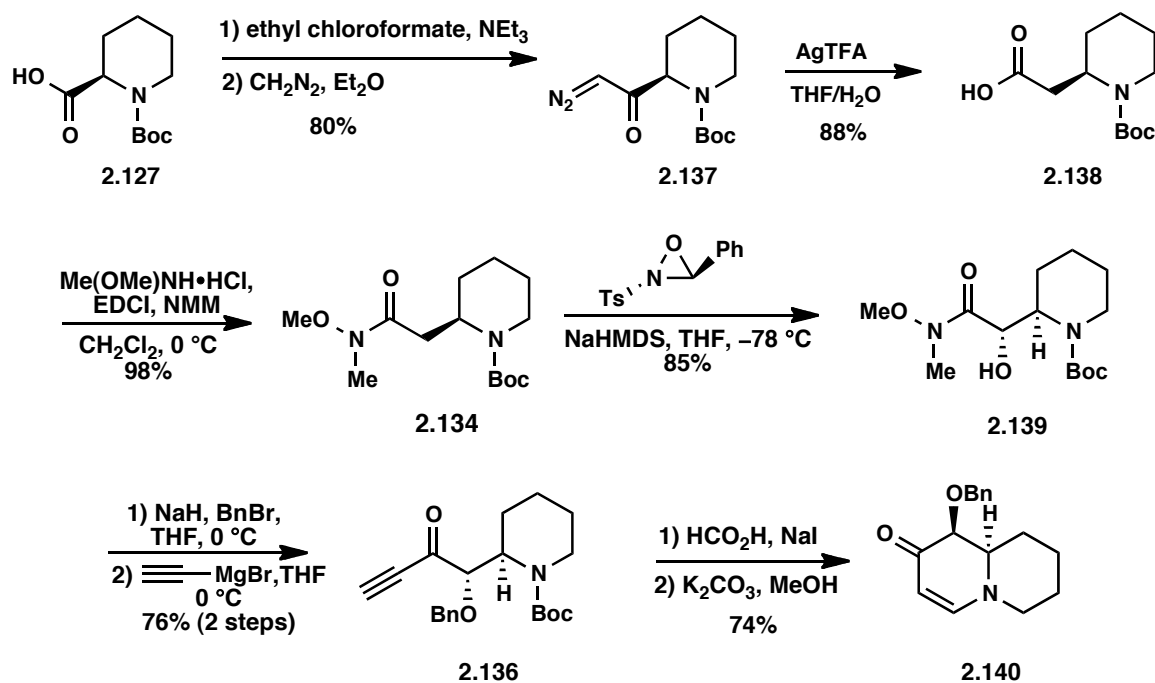
**Scheme 2-32.**  $\alpha$ -Oxygenation of  $\beta$ -amino ester **2.131** and  $\beta$ -amino amide **2.134**



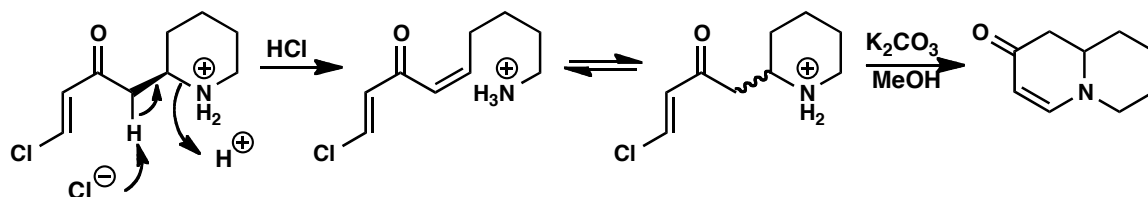
We next prepared ample amounts of  $\alpha$ -OH-6,6-enaminone **2.140** (Scheme 2-33). Starting from *N*-Boc-pipecolic acid (**2.127**), the diazoketone **2.137** was formed by reacting the mixed anhydride, formed from *N*-Boc-pipecolic acid and ethyl chloroformate, with diazomethane. This reaction was performed on a 50 mmol scale with excellent yields (ca 80%). The diazoketone was then subjected to Wolff rearrangement conditions in a mixture of THF/ $\text{H}_2\text{O}$ .<sup>313</sup> Under these conditions, a carbene is formed in the presence of a silver(I) catalyst; the carbene then rearranges to an electrophilic ketene, which reacted with water to form *N*-Boc-homopipecolic acid **2.138**. *N*-Boc-homopipecolic acid was converted to the Weinreb amide **2.139** using *N,O*-dimethylhydroxylamine and EDCI in excellent yield (98%). The  $\alpha$ -oxygenation proceeded smoothly as previously described. After protection of the alcohol as its benzyl ether, the  $\alpha$ -hydroxy amide was exposed to ethynylmagnesium bromide to form ynone **2.136** in 76% yield over two steps. Ynone **2.136** was then subjected to the cyclization

conditions previously discussed in Chapter 1. Since ynones have a propensity to racemize under Boc-deprotection conditions with 4M HCl/dioxane (Scheme 2.34) we used formic acid and NaI (external halide source) to mitigate racemization.<sup>47</sup> Thus, ynone **2.136** was first Boc-deprotected with formic acid and the corresponding vinyl iodide was formed with NaI, which was cyclized under basic conditions with K<sub>2</sub>CO<sub>3</sub> and MeOH to produce  $\alpha$ -OBn-6,6-enaminone **2.140**. The stereochemical outcome of the  $\alpha$ -oxidation was assigned at this stage using <sup>1</sup>H NMR coupling constants, which show a cis-relationship between the hydrogens.

**Scheme 2-33.** Synthesis of  $\alpha$ -OBn-6,6-enaminone **2.140**



**Scheme 2-34.** Potential acid catalyzed racemization mechanism



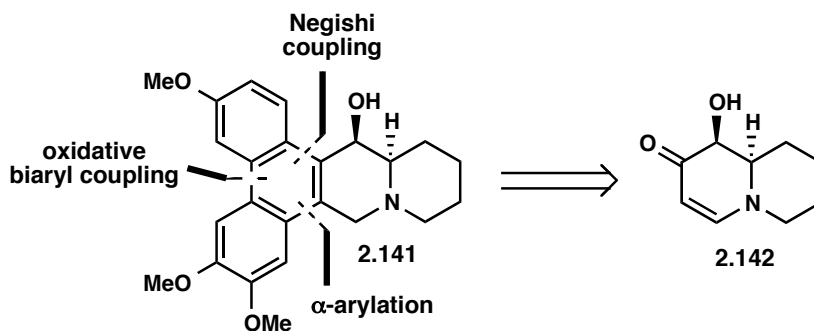
The protecting group for the hydroxyl group turned out to be quite important. The first choice was to use an acid labile protecting group such as the methoxymethyl ether (MOM) that could be removed under the acidic Boc-deprotection conditions. After protection as the MOM ether, the ynone was subjected to the acidic conditions that removed the Boc and MOM protecting groups, but the resultant  $\alpha$ -OH-vinyl iodide would not cyclize under the basic conditions. Similarly, not protecting the alcohol yielded the same result. When the alcohol was protected as the triethylsilyl ether (TES), the TES group inhibited the formation of the ynone from the Weinreb amide (e.g. **2.139**  $\rightarrow$  **2.136**). Eventual success was achieved when using acid and base stable groups such as the benzyloxymethyl ether (BOM) and the benzyl ether (Bn). The larger BOM protecting group and the smaller Bn produced approximately the same results when carrying them through to the protected 6,6-enaminone **2.140**.

At this point, we had the choice of two different routes to explore, both using chemistry previously developed in our laboratory.<sup>300,301</sup> The first route we chose was

more linear, with the key steps going forward being the Pd(II)-mediated  $\alpha$ -arylations discussed in Chapter 1 and the oxidative biaryl coupling of the northern and southern aryl

**Scheme 2-35.** Synthetic strategy towards the synthesis of (*R*)-15-Hydroxyboehmeriasin

A

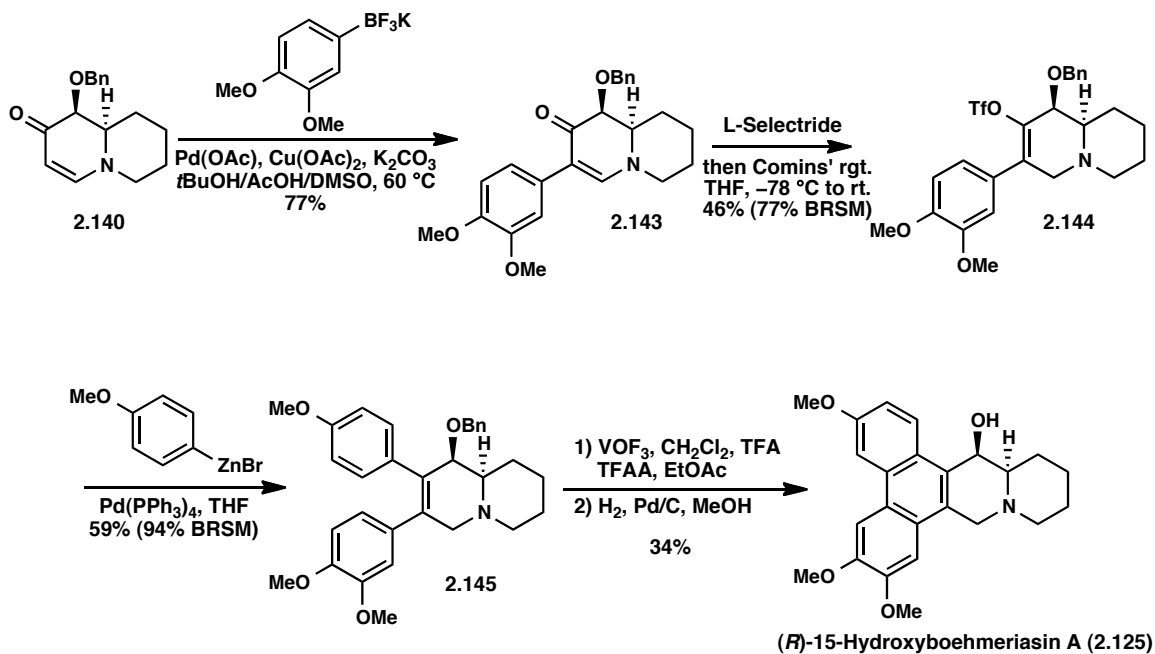


rings (Schemes 2-35). To explore this route, the protected 6,6-enaminone **2.140** underwent a Pd(II) catalyzed arylation with potassium 3,4-methoxyphenyltrifluoroborate to produce the  $\alpha$ -arylated enaminone **2.143** in good yield (Scheme 2-36). Next a 1,4-reduction of the  $\alpha$ -arylated enaminone **2.143** with L-Selectride and trapping of the resultant enolate with Comins' reagent gave triflate **2.144** in 40-50% yield, although the reaction never went to completion (77% BRSM). Given the previous experimental results (1,4-reduction of 6,6-enaminone without  $\alpha$ -oxygenation), it can be surmised from the half-chair conformation that the extra steric hindrance provided by the  $\alpha$ -oxygenation is impeding the reaction. With enough triflate **2.144** on hand, we attempted to the next reaction. A triflate can be used as a pseudohalide in various Pd-mediated coupling

reactions. A Negishi coupling was chosen, because the requisite aryl zincate could be prepared from the commercially available Grignard very easily and there had been previous success in coupling of sterically encumbered triflates.<sup>301</sup> Thus, stirring 4-methoxyphenylmagnesium bromide with anhydrous ZnBr<sub>2</sub> formed the zincate, which was added to a solution of triflate **2.144** and Pd(PPh<sub>3</sub>)<sub>4</sub> produced seco-boehmeriasin A **2.145** in 59% overall yield (94% BRSM). Previous experimental results had shown that the Negishi coupling of the  $\alpha$ -aryl-6,6-enaminone triflate provided the desired products within 1 hour at 60 °C in near quantitative yields.<sup>301</sup> This was not the case with triflate **2.144**. Elevated temperatures (up to 100 °C in DMA) and near stoichiometric quantities of Pd (0.7 equiv) failed to provide complete conversion. Even though the previous reactions did not provide adequate results in terms of yield, they did provide usable quantities of material. Seco-boehmeriasin A **2.145** was subjected to oxidative biaryl coupling conditions (VOF<sub>3</sub>) to join the northern and southern aryl fragments, however, in poor yield (38%). A significant amount of degradation was observed using this method. Other oxidants, such as PIFA<sup>314</sup> or FeCl<sub>3</sub><sup>315</sup> failed to provide any improvement (yields or amount of degradation). The benzyl group was removed using Pd/C under a H<sub>2</sub> atmosphere to afford hydroxyboehmeriasin A (**2.125**). It must be stressed that this route was inadequate in terms of yields, but gave us enough material along the way to complete the synthesis.



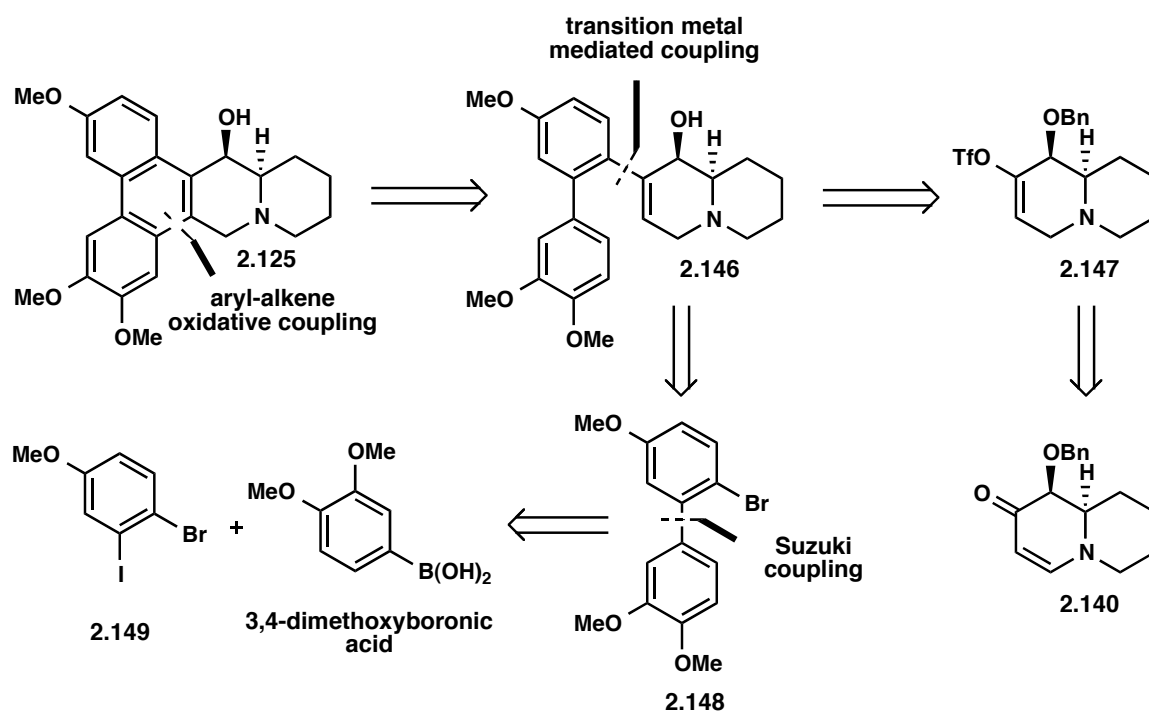
**Scheme 2-36.** First route toward (*R*)-15-Hydroxyboehmeriasin A



Not satisfied with the previous route, we pursued a more convergent route in *which* the key step would rely on a novel aryl-alkene oxidative coupling developed in our lab.<sup>300</sup> This route required the separate synthesis of the 6,6- $\alpha$ -oxygenated enaminone and the biaryl fragment (Scheme 2-37). The fragments would then be unionized, most likely via a transition metal mediated coupling. There would be several advantages to this route, first and foremost being a convergent route that cuts down the number of linear steps, theoretically, improving the overall yield of the synthesis. Second, there is flexibility in this route, specifically with the biaryl fragment that could be converted into a variety of coupling partners (i.e. zincate, Grignard, boronic acid/ester, stannane, etc...) given the

event one coupling partner fails to provide the desired results. Lastly, this route is amenable to library synthesis, which would allow for a systematic study of more polar phenanthropiperidines.

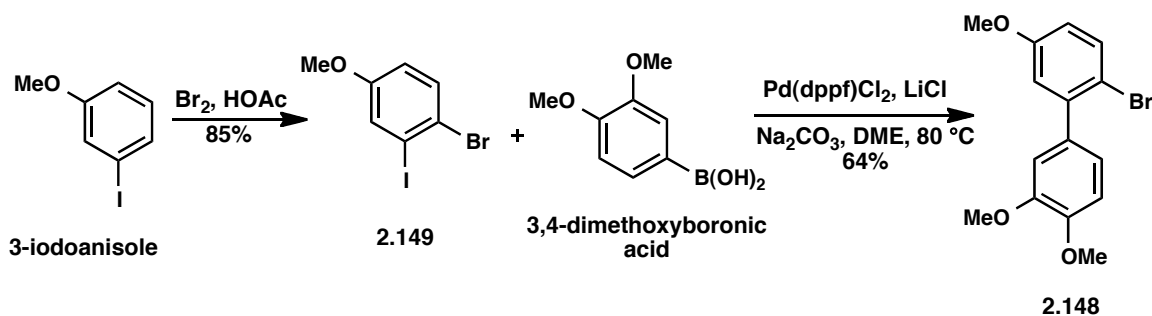
**Scheme 2-37.** Second synthetic plan towards the synthesis of (*R*)-15-Hydroxyboehmeriasin A



Having developed the chemistry to synthesize the key  $\alpha$ -oxidized-6,6-enaminone **2.140**, we turned our attention to the synthesis of the biaryl fragment **2.148** (Scheme 2-38). Fürstner and Kennedy have reported a straightforward route to prepare biaryl bromides that we were able to use.<sup>277</sup> 3-Iodoanisole was brominated with Br<sub>2</sub> and

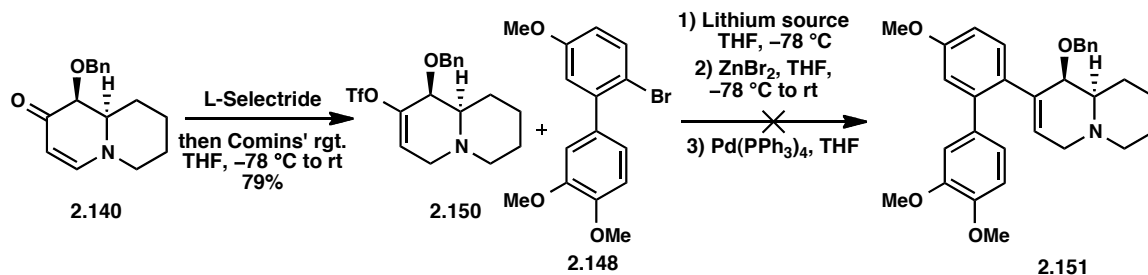
HOAc to give the 3-iodo-4-bromoanisole **2.149**. Next a Suzuki-Miyaura coupling with 3,4-dimethoxyboronic acid furnished the desired biaryl bromide **2.148** in 54% yield over two steps. This sequence was performed on a 25 g scale.

**Scheme 2-38.** Synthesis of biaryl fragment **2.148**



We then pursued the Pd-mediated coupling to join the two fragments.<sup>300</sup> To this end,  $\alpha$ -oxygenated-6,6-enaminone **2.140** was reduced with L-Selectride and the resultant enolate was trapped with Comins' reagent to afford triflate **2.150**. Biaryl bromide **2.148** was subjected to Li-halogen exchange conditions with either *n*-BuLi or *t*-BuLi in THF at

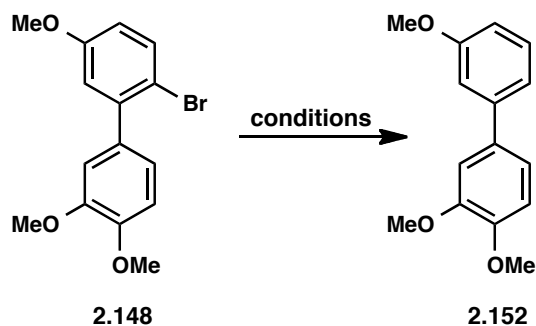
**Scheme 2-39.** Initial Negishi coupling attempt



$-78\text{ }^{\circ}\text{C}$ . The aryl lithium species was exposed to anhydrous  $\text{ZnBr}_2$  to form the appropriate zincate. Triflate **2.150** and  $\text{Pd}(\text{PPh}_3)_4$  were added to the zincate which did not produce any of the desired product. It was clear from the reaction that the problem was either the Li-halogen exchange or the zincate formation, because an almost quantitative amount of triflate **2.150** was recovered. At this point we thought the problem was fairly obvious, namely, that water was interfering with the reaction. The first source of water investigated was the biaryl bromide **2.148** (Table 2-14). This seemed like a likely candidate because the biaryl bromide was an oil under reduced pressure, but as soon as it was exposed to air it started solidifying. This may have trapped water molecules within the crystal lattice. The solution to the unwanted addition of water was to azeotrope the biaryl with benzene or toluene. Unfortunately, this had no effect on the lithium halogen exchange because a  $^1\text{H}$  NMR of the Li-halogen exchange showed only partial exchange (ratio of exchanged to non-exchanged product was 1:1). In fact, complete exchange was only observed when  $>10$  equivalents of lithium source were used. The next source investigated was the solvent, because THF is miscible with water and water can be difficult to remove. Again, all attempts at drying the THF<sup>316</sup> (alumina, silica, activated  $3\text{ \AA}$  molecular sieves) failed to provide complete exchange, although the ratio of exchange

to non-exchanged product was higher (3:1, table 2-14 entries 1, 3, and 4). Ether was also tried as a solvent, but the biaryl bromide was not soluble at low temperatures (Table 2-14, entry 2). Another commonly employed technique is to add *N,N,N',N'*-tetramethylethylenediamine (TMEDA) to break up any lithium aggregates in solution, but that approach did not provide any exchanged product (Table 2-14, entry 5). In all likelihood, the issue of water interfering in the reaction is a combination of a number of factors, including the factors previously discussed. One way to possibly get around this issue was to form another coupling partner. The zincate could be formed from the Grignard or may be used directly in the coupling reaction. To this end,  $\text{Mg}^0$  was used with  $\text{I}_2$  or DIBAL-H as an activator (Table 2-14, entries 6 and 7; again, these experiments only provided partially exchanged material.

**Table 2-14.** Attempts at exchanging bromine for hydrogen



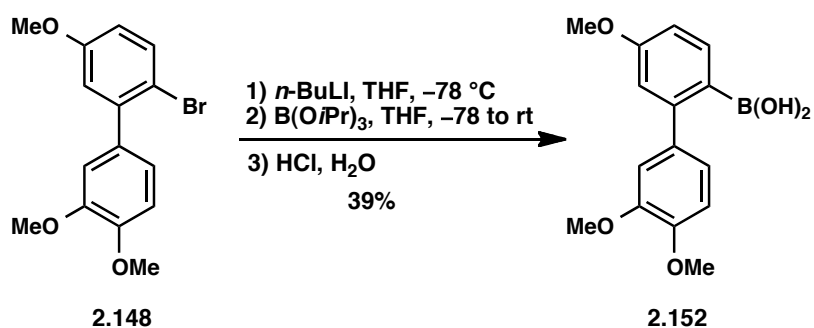
| entry | solvent          | exchange source | temperature(°C) | additive       | result <sup>a</sup> |
|-------|------------------|-----------------|-----------------|----------------|---------------------|
| 1     | THF <sup>b</sup> | <i>t</i> -BuLi  | -78             | -              | partial exchange    |
| 2     | ether            | <i>t</i> -BuLi  | -78             | -              | no exchange         |
| 3     | THF <sup>c</sup> | <i>n</i> -BuLi  | -78             | -              | partial exchange    |
| 4     | THF <sup>d</sup> | <i>t</i> -BuLi  | -78 to rt       | -              | partial exchange    |
| 5     | THF              | <i>t</i> -BuLi  | -78             | TMEDA          | no exchange         |
| 6     | THF              | Mg <sup>0</sup> | rt              | I <sub>2</sub> | partial exchange    |
| 7     | THF              | Mg <sup>0</sup> | rt              | DIBAL-H/LiCl   | partial exchange    |

<sup>a</sup> determined by <sup>1</sup>H NMR after acidic quench. <sup>b</sup> Dried via passing over a neutral alumina column (10% m/v). <sup>c</sup> Dried via passing over a silica gel column (230-400) mesh, 10% m/v. <sup>d</sup> Dried over 3Å molecular sieves (20% m/v).

The next approach was to synthesize a coupling partner that could be isolated (e.g., boronic acid/ester or aryl stannane). The most obvious choice was to make the boronic acid. Although a Li-halogen exchange is still required for the synthesis, this method allows the coupling partner to be isolated and purified. Thus, the Li-halogen exchange was performed (scheme 2-40). The aryl-lithium species was reacted with tri-isopropyl borate and hydrolyzed with 10% HCl to yield boronic acid **2.152**. One issue that concerned us was that the purification of the boronic acids can be very extensive. This was not the case for the purification of boronic acid **2.152**, in which two purification procedures were needed to obtain pure material (one column chromatographic separation and one recrystallization). One purification operation was insufficient because after

column chromatography, impurities still remained as evidenced by the  $^1\text{H}$  NMR and the boronic acid would not crystallize before column purification. As shown in Scheme 2-40, the lithium halogen exchange is still an issue as evident by the low yields, however this could be performed on large scale (5 g) to produce ample amounts of boronic acid **2.152**.

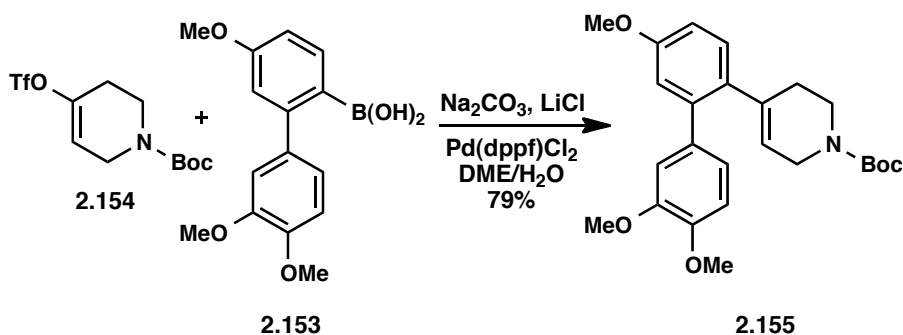
**Scheme 2-40.** Synthesis of boronic acid



The availability of boronic acid **2.152** provided us with additional flexibility in our synthetic route. There are several potential problems with traditional Suzuki couplings using boronic acids such as protodeboronation, homo-coupling, and polymerization leading to by-product formation, if there indeed is any reaction at all without using high catalyst loading. These issues can be avoided or minimized using either trifluoroborates<sup>317</sup> or MIDA<sup>318</sup> boronates. In many ways, they can be viewed as protected boronic acids because a mild aqueous base is generally needed to release the reactive coupling species. Boronic acid **2.153** was first used in a test reaction (Scheme 2-41). Commercially available *N*-*boc*-4-piperidone was treated with LHMDS to form the

enolate, which was trapped with Comins' reagent to form triflate **2.154**. Triflate **2.154** was then used as the coupling partner with biaryl boronic acid **2.153** in the Suzuki coupling. Under standard coupling conditions (mild aqueous base, Pd(0), solvent/H<sub>2</sub>O) the reaction proceeded with excellent yield and was complete within 1 hour to furnish the aryl coupling precursor **2.155**. We next carried out the reaction with the target substrate

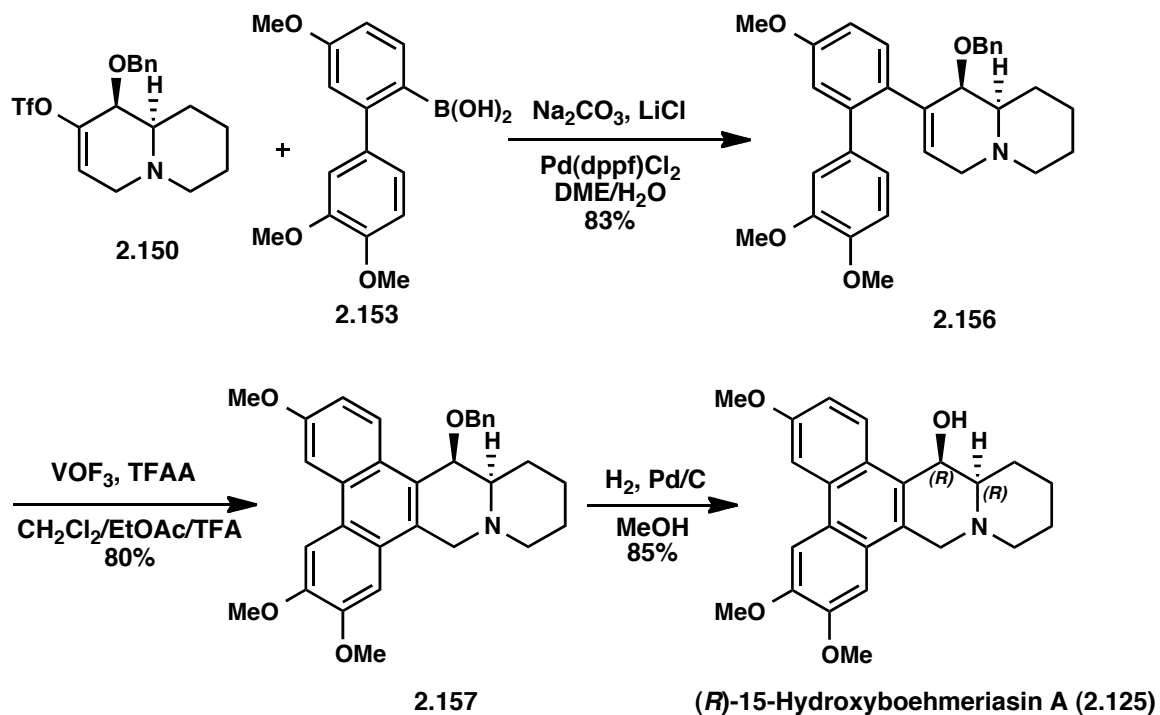
**Scheme 2-41.** Test coupling with boronic acid coupling partner



(Scheme 2-42) and found that substrate **2.150** worked even better (83% yield) than the test case and in a shorter period of time (30 min). The key aryl-alkene oxidative coupling was carried out with a fresh solution of VOF<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub>/EtOAc/TFA/TFAA to provide protected hydroxyboehmeriasin A **2.157** in 80% yield. The deprotection of the benzyl group, accomplished using Pd/C under a H<sub>2</sub> atmosphere, provided hydroxyboehmeriasin A (**2.125**) in 85% yield. The synthesis of hydroxyboehmeriasin A was completed in 11 steps with a 15% overall yield. Hydroxyboehmeriasin A (**2.125**) currently is being tested in various *in vitro* assays.



**Scheme 2-42.** Completion of the synthesis of (*R*)-15-Hydroxyboehmeriasin A



#### 2.4.2 Biological activity of polar phenanthropiperidines

With the synthesized hydroxyboehmeriasin A in hand, we wanted to test its antiproliferative activity in cancer cell lines. Much to our delight, hydroxyboehmeriasin A was active in A549 cells with a  $\text{GI}_{50}$  of 81 nM. This is very encouraging because with the addition of the hydroxy functionality we have a functional handle to append other water solubilizing moieties that may further aid in the *in vitro* and *in vivo* activity. Further studies are underway to assess the antiproliferative activity against other cell lines

(colo-205 and NCI-ADR-RES) and its activity in the NF- $\kappa$ B functional assay to possibly assess a tentative mode of action.

**Table 2-15.** Antiproliferative activity of hydroxyboehmeriasin A against A549 cells

| compound              | GI <sub>50</sub> (nM) <sup>a</sup> |
|-----------------------|------------------------------------|
| (±)-tylophorine       | 45                                 |
| (S)-tylophorine       | 10                                 |
| hydroxyboehmeriasin A | 81                                 |

<sup>a</sup>A549/NF- $\kappa$ B-luc= human lung carcinoma.

### 2.4.3 Summary of polar phenanthropiperidines

The failure of tylocrebrine in the clinic has established a potential liability for this class of alkaloids and their use in the clinic. Previous studies in our research group aimed at finding the underlying mechanism for the observed ataxia and disorientation in patients provided inconclusive results. We therefore pursued a strategy that avoids those undesirable side effects by designing analogues that cannot enter the central nervous system. This approach does not require an understanding of the mechanisms of the side effects. To address the issue, we adopted a strategy to add polar functionality to the parent compound. Based on previous SAR studies we hypothesized that a C15 hydroxyl group could be added to Boehmeriasin A without reduction in antiproliferative activity. We devised a synthetic route that provided (*R*)-15-Hydroxyboehmeriasin A in 11-steps

with a 15% overall yield. The key transformations involved an  $\alpha$ -oxygenation and an aryl-alkene oxidative coupling. (*R*)-15-Hydroxyboehmeriasin A was tested against the A549 lung carcinoma cell line, in which it showed a GI<sub>50</sub> of 81 nM. Current studies are underway to further ascertain the biological viability of (*R*)-15-Hydroxyboehmeriasin A including against multidrug-resistant cell lines as well as a tentative mechanism of action study.

## Chapter 3

### Total Synthesis of (±)-Dihydrolyfoline

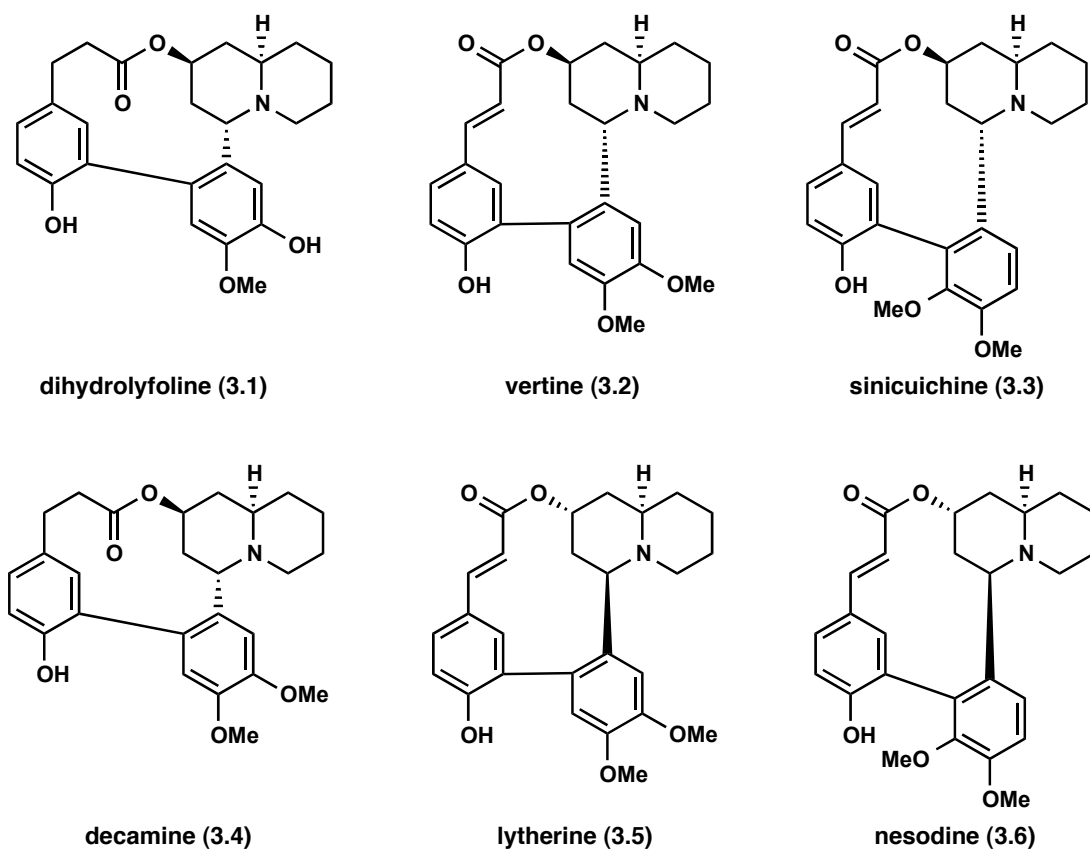
#### 3.1 Introduction

Synthetic methodology is often limited in value until it can be shown to have some practical applications. As discussed before, previous work has shown that a variety of cyclic enaminones could be elaborated into phenanthroindolizidines and phenanthroquinolizidines. Herein, we demonstrate that the 6,6-enaminone can be used as a precursor to (±)-dihydrolyfoline **3.1** (Figure 3-1); a representative member of the biphenylquinolizidine lactone alkaloid family. This natural product was not only chosen because of its structure, but also due to the family's multitude of biological properties.

#### 3.2 Structure and isolation

Extracts from the Lythracea family of plants have been known and used in medicine since the early 1600's.<sup>319</sup> The first alkaloids were isolated from the Lythraceae plant family in 1962.<sup>320</sup> There have now been more than 20 alkaloids isolated from this plant family (Figure 3-1). Early structural studies<sup>321</sup> established the presence of a cinnamic lactone or its dihydro-equivalent, three aromatic oxygens, two aromatic nuclei, and a tertiary nitrogen. The biphenylquinolizidine lactone alkaloids are considered the primary biologically active components of mature *Heimia*, one of the genera of the Lythracea family. The pharmacological properties of these alkaloids are not fully known,

but some of their properties have been clinically studied.<sup>322</sup> The most abundant alkaloid in *H. salicifolia* is vertine (**3.2** also known as cryogenine),<sup>323</sup> which is most often considered as being the primary source for the effects of the traditional *Heimia salicifolia* extract. Clinically observed effects of vertine include anti-cholinergic, anti-inflammatory, anti-spasmodic, hyperglycemic, hypotensive, sedative, tranquilizer, and vasodilator activity.<sup>322,324</sup> Lythrine (**3.5**) is considered to be the most effective of the family of alkaloids. Lythrine and the mixed alkaloidal extract are considered to be relatively free of toxicity.<sup>322</sup> Nesodine (**3.6**) has been shown to produce an anti-inflammatory effect, and sinicuichine (**3.3**) is known to act as a tranquilizer.<sup>319,322</sup>



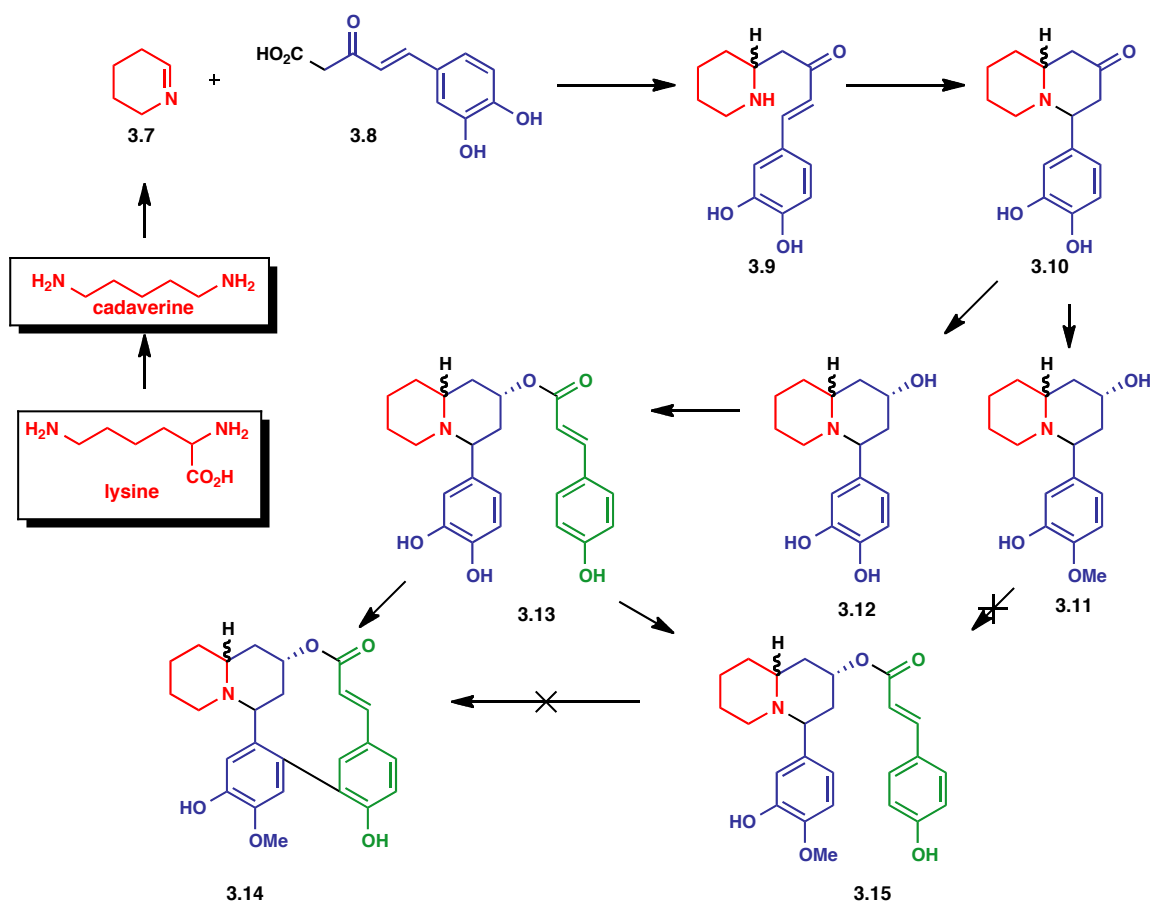
**Figure 3-1.** Structure of selected biphenylquinolizidine lactone alkaloids.

### 3.3 Biosynthesis of biphenylquinolizidine lactone alkaloids

The biosynthesis of this class of alkaloids has been extensively studied. Independent investigations by Herbert and Spenser have shown that lysine is incorporated into the natural products through the intermediacy of cadavarine.<sup>325-327</sup> Labeling experiments have also shown that phenylalanine precursors are incorporated into both the quinolizidine ring, as well as into the coumaric ester.<sup>321,328</sup> The biosynthetic

scheme is shown in Scheme 3-1.<sup>329</sup> The cyclic imine **3.7**, formed from cadaverine, undergoes a condensation reaction with acid **3.8** to give piperidine **3.9**. Piperidine **3.9** then reacts to furnish quinolizidinone **3.10** (cis and trans products). The level of

**Scheme 3-1.** Biphenylquinolizidine lactone alkaloid biosynthesis



hydroxylation at which the condensation between the lysine- and phenylalanine-derived units takes place has not been unequivocally established. What is known is that both the cis- and trans- quinolizidinone **3.10** are effective precursors of the natural products.<sup>325,326</sup>

Quinolizidinone **3.10** is further reduced to yield quinolizidinols **3.11** and **3.12**. Quinolizidinol **3.11** is demethylasubine I or II (depending on the stereochemistry) and is not converted into any of the biphenylquinolizidine lactone alkaloids. Quinolizidinol **3.12** is esterified with coumaric acid to the coumaric ester **3.13**. Coumaric ester **3.13** is transformed into either the biphenylquinolizidine lactone alkaloids **3.14** or the phenylquinolizidine ester alkaloids **3.15**. Interestingly, it has been proposed that phenylquinolizidine ester alkaloids serve as precursors to the biphenylquinolizidine lactone alkaloids, but has not been proven to date.<sup>328</sup>

### **3.4 Synthesis of biphenylquinolizidine lactone alkaloids**

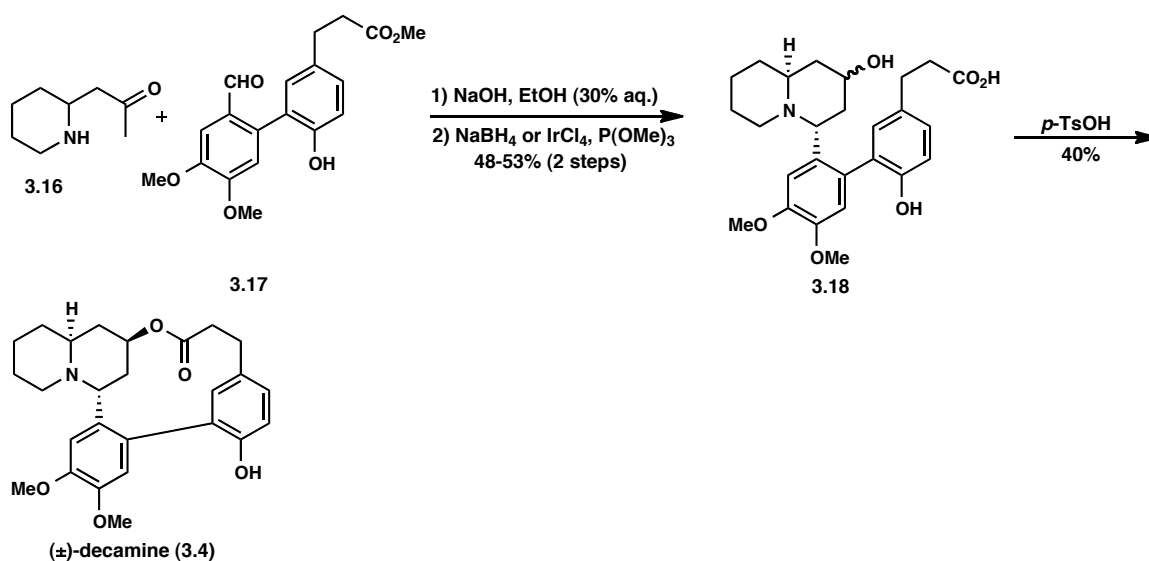
Only a few syntheses of these alkaloids have appeared in the literature, despite that fact that many studies have been performed to elucidate their biosynthesis and to investigate their pharmacological properties. The few syntheses that have been published are widely dispersed, with the first being published in 1977<sup>330</sup> and the last in 2012.<sup>331</sup> As discussed in previous chapters, the key steps in each synthesis will be discussed, which in most cases, concern the macrocyclization strategy.

The first natural product prepared in 1977 by chemical synthesis was ( $\pm$ )-decamine (**3.4** Scheme 3-2).<sup>330</sup> This synthesis features the condensation of pelletierine (**3.16**) with biphenylcarboxaldehyde **3.17** under weakly basic conditions to yield the quinolizidinone, which was further reduced with either NaBH<sub>4</sub> or IrCl<sub>4</sub> and P(OMe)<sub>3</sub> to give the quinolizidinol **3.18**. Employing aqueous THF in the condensation step produced a 3:2 mixture of cis/trans isomers while reaction in MeOH yielded only the trans-isomer.



The *cis*-isomer was obtained using a 30% aqueous EtOH solution in 53% yield. Reduction of the quinolizidinone with NaBH<sub>4</sub> delivers the hydride into the sterically less hindered convex face of the molecule, while IrCl<sub>4</sub> uses that site for complexation and the hydride is delivered to the concave face. A high dilution *p*-TsOH catalyzed macrolactonization step produced (±)-decamine (**3.4**) in 40% yield.

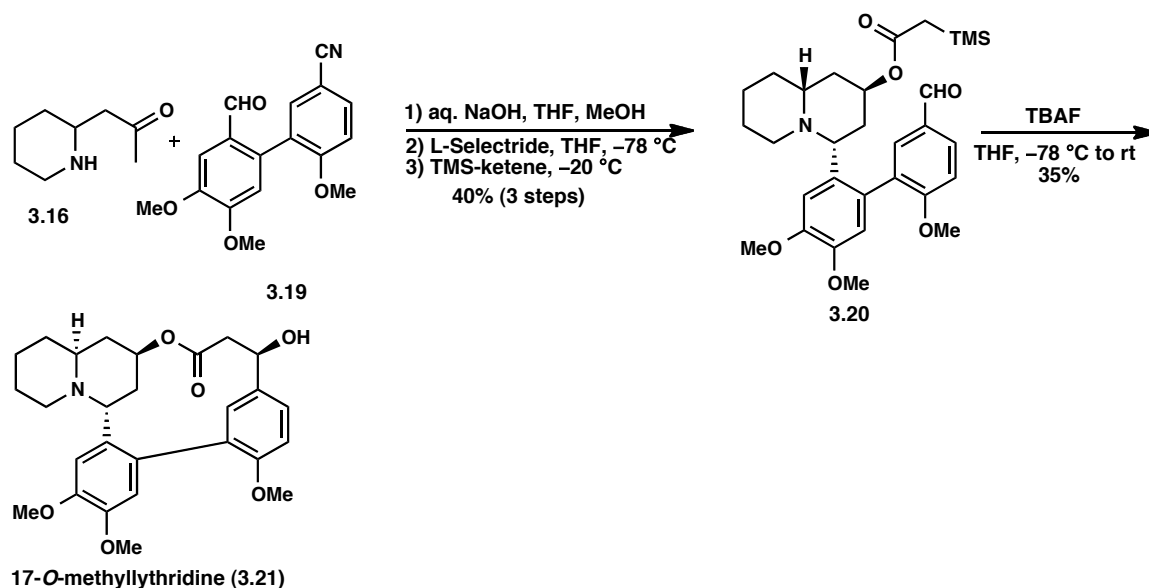
**Scheme 3-2.** Synthesis of (±)-decamine (**3.4**)



In a synthesis of 17-*O*-methyllythridine, Seitz *et al.* took a unique approach using a TMS-acetate as the macrocyclization precursor (Scheme 3-3).<sup>332</sup> Similar to the previous synthesis, pelletierine (**3.16**) is condensed with biaryl-aldehyde **3.19** to give the quinolizidinone, which is reduced to the quinolizidinol with L-Selectride and acylated with TMS-ketene to produce TMS-acetate quinolizidine **3.20**. TMS-acetate quinolizidine

**3.20** is treated with TBAF, which is desilylated, and then undergoes an intramolecular aldol reaction with the aldehyde moiety to yield 17-*O*-methyllythridine (**3.21**). The macrocyclization event is relatively low yielding at 35%. The major side product is the uncyclized desilylated derivative of **3.20**.

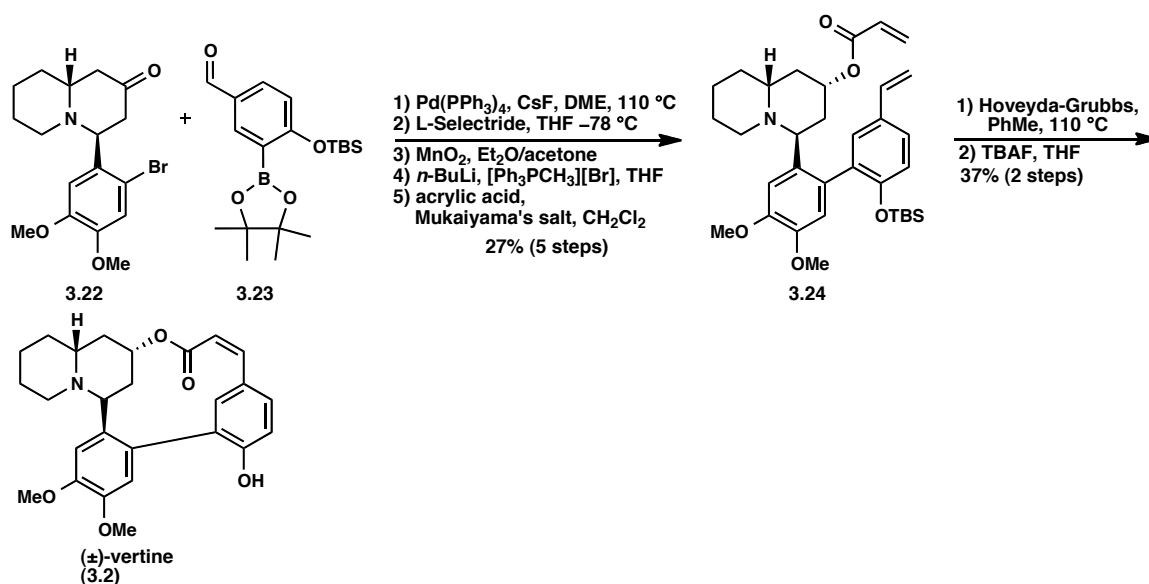
**Scheme 3-3.** Macrolide closure via fluorodesilylation and aldol reaction



The most recent total synthesis of ( $\pm$ )-vertine (Scheme 3-4) by Chausset-Boissarie and coworkers used a *Z*-selective RCM as the macrocyclization key step.<sup>333</sup> The beginning of the synthesis is quite similar to previous approaches using the condensation of *N*-Boc-pelletierine with bromoverataldehyde to generate quinolizidinone **3.22**. After a

Suzuki coupling with the appropriate arylboronic ester **3.23** and several standard transformations, they arrived at the cyclization precursor **3.24**. After extensive experimentation, it was discovered that the Hoveyda-Grubbs catalyst in toluene at refluxing temperatures was optimal for the Z-selective RCM to achieve the first total synthesis of (±)-vertine **3.2**.

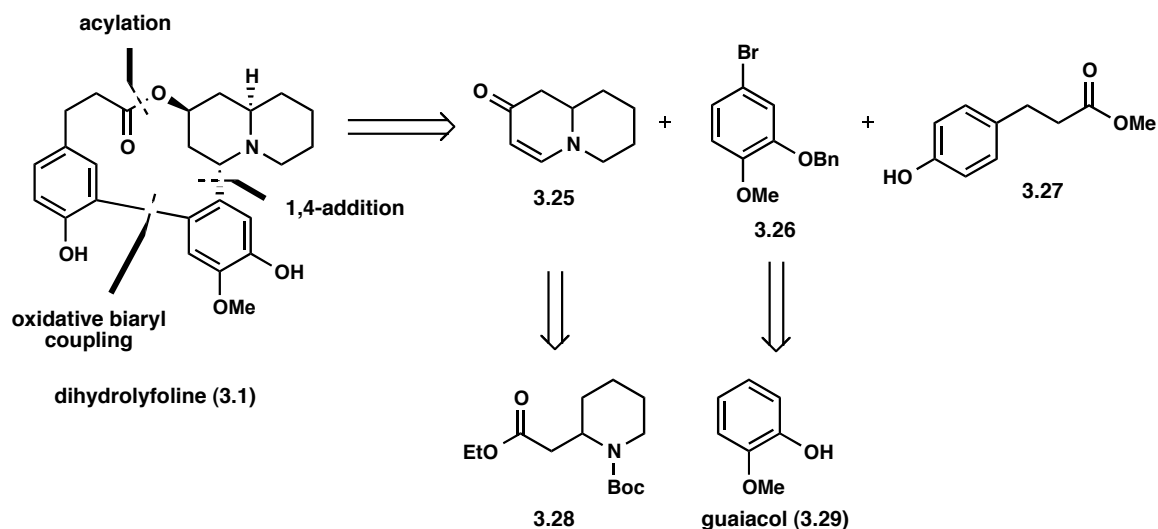
**Scheme 3-4.** Z-selective RCM approach in the synthesis of (±)-vertine



### 3.5 Total synthesis of (±)-dihydrolyfoline

Our total synthetic strategy for dihydrofoline (**3.1**) involved the further elaboration of the VOF<sub>3</sub> oxidative coupling shown in the previous chapter. This extraordinary biaryl coupling would be the

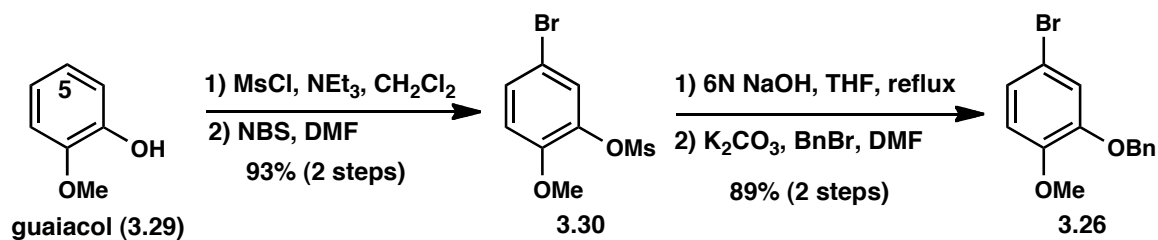
**Scheme 3-5.** Retrosynthetic analysis of ( $\pm$ )-dihydrolyfoline



macrocyclization event leading to the natural product. This type of cyclization has been shown once before to form a twelve-membered lactam in Evans' biomimetic synthesis of vancomycin.<sup>334</sup> To this end our retrosynthetic analysis is shown in Scheme 3-5. Three disconnections of ( $\pm$ )-dihydrolyfoline show that the natural product can be broken into three separate building blocks: 6,6 enaminone **3.25**, aryl bromide **3.26**, and dihydrocinnamic ester **3.27**. The enaminone can be made from homopipercolic ester **3.28**, while the aryl bromide is synthesized from commercially available guaiacol (**3.29**).

Since an established route was available for the synthesis of the 6,6-enaminone (**3.25**), we focused on preparing aryl bromide **3.26** (Scheme 3-6). Starting from guaiacol (**3.29**) the phenol was reacted with methanesulfonyl chloride to produce the corresponding aryl mesylate. Bromination with NBS specifically brominates the C5 position giving aryl bromo mesylate **3.30**.<sup>335</sup>

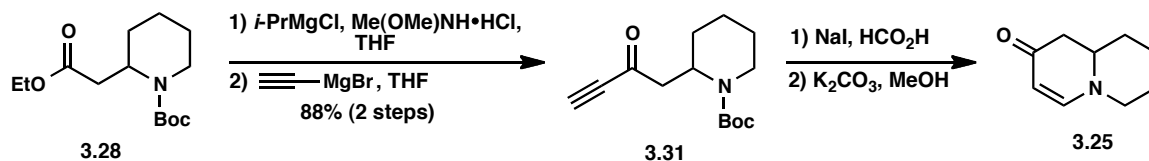
**Scheme 3-6.** Synthesis of aryl bromide **3.26**



When guaiacol is brominated directly, the bromination occurs at the C4 position, not the C5 position.<sup>336</sup> Therefore, the electronics of the system had to be changed to an electron withdrawing directing group to brominate the C5 position. These two reactions provide insights into the biaryl coupling reaction we are planning to use for the macrocyclization. It may be inferred that the oxidative biaryl coupling needs an unprotected phenol or an electron donating directing group to couple at the desired position of the aryl rings. One element, in our favor, is that in both aryl rings directing groups are present at the correct positions. From here, removal of the mesyl-directing group with 6N NaOH provided the phenol, which was immediately protected as its benzyl ether with BnBr. This four-step sequence was extremely high yielding at 83%. Moreover, this four-step sequence could be performed on a very large scale (>15g) with a single recrystallization after the last step.

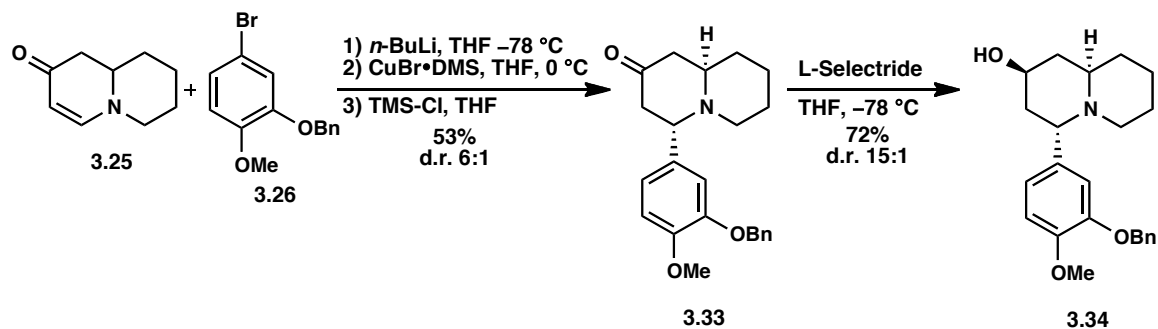
The 6,6-enaminone **3.25** was prepared from as previously described from homopipercolic ester **3.28** by transformation to the Weinreb amide by reaction with *N,O*-dimethylhydroxyl amine and *i*-PrMgCl, which was then reacted with ethynylmagnesium bromide to give ynone **3.31**. This ynone was subjected to the one-pot deprotection cyclization conditions discussed previously.

**Scheme 3-7.** Synthesis of 6,6-enaminone **3.25**



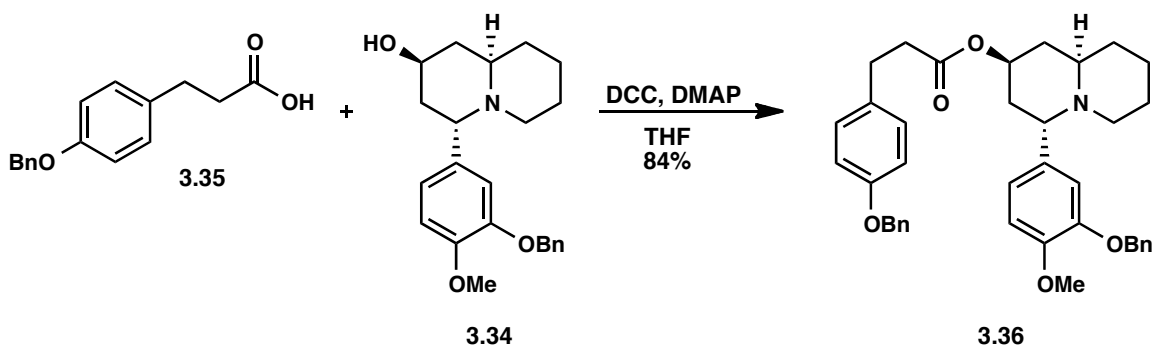
The next goal of the synthesis involved the elaboration of the 6,6-enaminone to (±)-dihydrolyfoline as shown in Scheme 3-8. The first step was the substrate controlled 1,4-addition. This reaction was diastereoselective due to the butterfly shape of the enaminone. To allow for the conjugate addition to occur, a soft metal complex needed to be formed. To this end, a lithium halogen exchange was performed with aryl bromide **3.26**, and the resultant lithium intermediate was exchanged with CuBr•DMS to form the

**Scheme 3-8.** Synthesis of quinolizidinol **3.34**



corresponding cuprate. A solution of the enaminone was added to the cuprate, which formed the desired quinolizidine **3.33** with moderated diastereoselectivity. Quinolizidine **3.33** was then reduced with L-Selectride to produce quinolizidinol **3.34** with very good diastereoselectivity. Acylation of quinolizidinol **3.34** (Scheme 3-9) with acid **3.35**, synthesized in two steps from commercially available material, proceeded smoothly affording phenyl quinolizidine ester **3.36**.

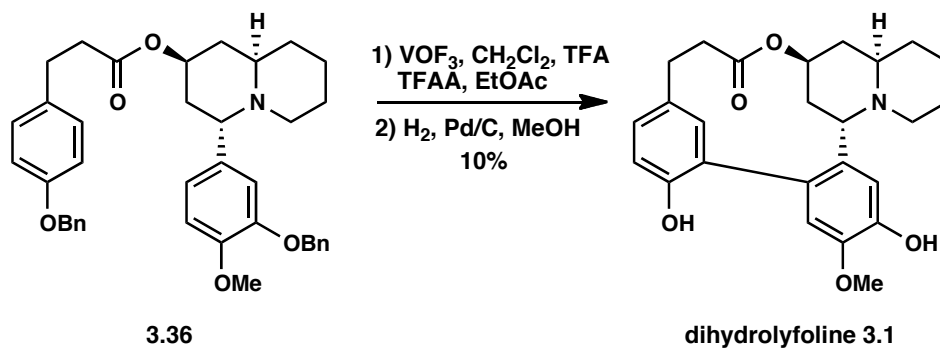
**Scheme 3-9.** Synthesis of phenyl quinolizidine ester **3.36**



Our group has been interested in the oxidative coupling of aryl systems, as well as aryl-alkene systems for some time. In fact, they have been used in several total syntheses from our group.<sup>300,301</sup> This is an intriguing aspect to the synthesis of dihydrolyfoline, and in fact biosynthetic studies (Scheme 3-1) have shown that this transformation is the final step in the biosynthesis of this class of alkaloids. Thus, for the penultimate key step of the synthesis we proposed an oxidative biaryl coupling to construct the 12-membered macrolactone. This type of macrocyclization is relatively underexplored and could provide unique disconnections that have not used before if effective reaction conditions

can be found. Our initial studies have shown promising results (10% yield with recover of 50% of starting material), however this reaction will take some optimization. To our knowledge this will be the first total synthesis of this natural product.

**Scheme 3-10.** Oxidative biaryl coupling forming dihydrolyfoline (**3.1**)



### 3.6 Summary

In an effort to expand the use of the 6,6-enaminone as a building block for nitrogen containing heterocycles in target molecules or natural products, we have embarked on a total synthesis of dihydrolyfoline, a representative member of the biphenylquinolizidine lactone alkaloid family. The synthesis can be accomplished in short order (5-steps) from the 6,6-enaminone **3.25**. Given our interest in the oxidative coupling of aryl systems, we chose to use this coupling as the key macrocyclization step in the synthesis of the natural product. While the yield is low, our proof of concept shows that this type of macrocyclization can be accomplished. The sheer simplicity of the



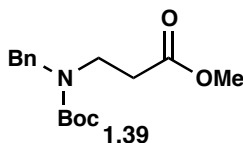
enaminone suggest that the enaminone can be used as a building block for a wide range of target molecules and dihydrolyfoline is no exception. The reactions developed along the way to synthesizing this natural product are a contribution to a growing arsenal of wide ranging organic reactions.

## **Chapter 4 Experimental Data**

## 4.1 Materials and Methods

Unless otherwise specified, all starting materials, reagents, and solvents are commercially available and were used without further purification. Flash column chromatography was carried out on silica gel. TLC was conducted on silica gel 250 micron, F254 plates.  $^1\text{H}$  NMR spectra were recorded on a 400 MHz NMR instrument. Chemical shifts are reported in ppm with TMS or  $\text{CHCl}_3$  as an internal standard (TMS: 0.00 ppm,  $\text{CHCl}_3$ : 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), integration and coupling constants (Hz).  $^{13}\text{C}$  NMR spectra were recorded on a 100 MHz NMR spectrometer with complete proton decoupling. Chemical shifts are reported in ppm with the solvent as internal standard ( $\text{CHCl}_3$ : 77.2 ppm). IR spectra of solids were obtained by dissolving the sample in  $\text{CHCl}_3$  and letting the solvent evaporate on a KBr plate. High-resolution mass spectrometry was performed by the University of Minnesota Mass Spectrometry Facility.

## 4.2 Chapter 1

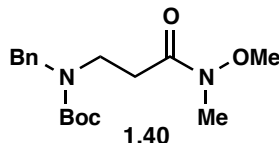


*Methyl 3-(Benzyl(tert-butoxycarbonyl)amino)propanoate (1.39).*

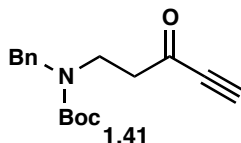
Method A: A 100 mL round-bottomed flask was charged with benzylamine (4.0 mL, 37 mmol, 1.5 equiv) and cooled to  $-40\text{ }^\circ\text{C}$  under a  $\text{N}_2$  atmosphere. Methyl acrylate (2.20 mL,

25 mmol, 1.00 equiv) was added dropwise over 5 min and the reaction was stirred at  $-40$  °C for 48 h. Excess benzylamine was distilled off under reduced pressure. The remaining residue was dissolved in MeOH (50 mL) and di-*tert*-butyldicarbonate (6.40 g, 29.3 mmol, 1.20 equiv) was added slowly. The reaction mixture was stirred for another 30 min and the solvent was removed *in vacuo*. The concentrated reaction mixture was re-dissolved in CH<sub>2</sub>Cl<sub>2</sub> (300 mL) and washed with cold 10% HCl (100 mL x 2) and brine (100 mL x 2). The organic layer was dried with MgSO<sub>4</sub>, concentrated, and purified via SiO<sub>2</sub> flash column chromatography (25% EtOAc/hexanes) to afford 6.62 g (88%) of the methyl ester as a clear viscous oil.

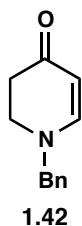
Method B: Under a nitrogen atmosphere, a solution of methyl acrylate (13.63 mL, 151.5 mmol, 1.00 equiv) was added via an addition funnel to a stirring mixture of benzylamine (16.63 mL, 151.5 mmol, 1.00 equiv) and MgBr<sub>2</sub>•OEt<sub>2</sub> (11.78 g, 45.62 mmol, 0.30 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (100 mL) in a 500 mL round bottom flask under a N<sub>2</sub> atmosphere. The reaction was stirred at rt until completion, as determined by TLC (~24 h). The reaction mixture was quenched with H<sub>2</sub>O and the organic layer was separated and concentrated under reduced pressure to give a colorless oil. The oil was dissolved in a minimal amount of MeOH (~50-75 mL) and di-*tert*-butyldicarbonate (39.82 g, 182.4 mmol, 1.20 equiv) was added slowly. The reaction mixture was stirred for another 30 min and the solvent was removed *in vacuo*. The concentrated reaction mixture was re-dissolved in CH<sub>2</sub>Cl<sub>2</sub> (300 mL) and washed with cold 10% HCl (100 mL x 2) and brine (100 mL x 2). The organic layer was dried with MgSO<sub>4</sub>, concentrated and purified via SiO<sub>2</sub> flash column chromatography (25% EtOAc/hexanes) to afford 41.25 g (92%) of the methyl ester as a clear viscous oil. Spectral data for this compound was identical to that reported in the literature.<sup>47,337</sup>



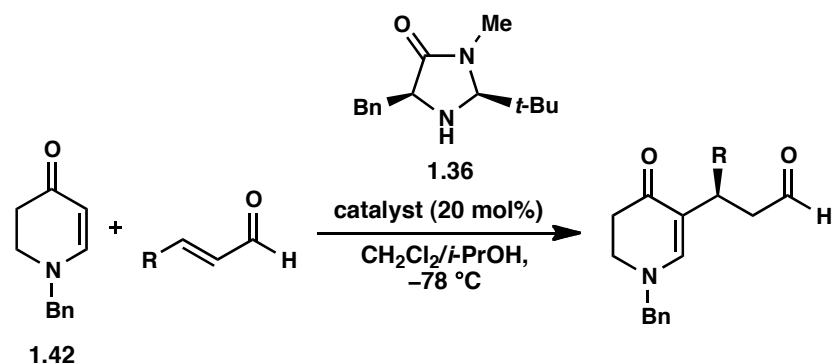
*tert-Butyl Benzyl(3-(methoxy(methyl)amino)-3-oxopropyl)carbamate (1.40)*. Methyl 3-(Benzyl(*tert*-butoxycarbonyl)amino)propanoate (**1.39**, 20.56 g, 70.17 mmol, 1.00 equiv) and *N,O*-Dimethylhydroxylamine (10.63 g, 108.9 mmol, 1.55 equiv) were slurried in THF (100 mL) in a 500 mL round bottom flask under a N<sub>2</sub> atmosphere and cooled to -20 °C. A solution of isopropylmagnesium chloride (105 mL, 210 mmol, 3.00 equiv) was added dropwise maintaining a temperature below -10 °C. Upon completion (~1 h), as judged via TLC, the reaction was quenched with sat. aq. NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O (3x), dried (MgSO<sub>4</sub>), concentrated under reduced pressure, and purified via SiO<sub>2</sub> flash column chromatography (40% EtOAc/hexanes) to afford 19.90 g (88%) of the Weinreb amide as a colorless oil: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (1:1 mixture of rotamers) 1.44 (s, 9H), 1.50 (s, 9H), 2.58-2.63 (bm, 2H), 2.67-2.72 (bm, 2H), 3.15 (s, 6H), 3.43-3.48 (bm, 2H), 3.51-3.55 (bm, 2H), 3.63 (bs, 6H), 4.48 (s, 4H), 7.22-7.35 (m, 10H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 28.4, 31.2, 32.1, 42.8, 43.1, 50.6, 51.6, 61.2, 61.3, 79.8, 127.2, 127.3, 127.8, 128.4, 138.4, 138.8, 155.5, 155.8, 172.5, 172.9; IR (neat) 2974, 1693, 1664, 1413, 1366, 1167 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>17</sub>H<sub>27</sub>N<sub>2</sub>O<sub>4</sub>: 323.1971, found 323.1957.



*tert*-Butyl Benzyl(3-oxopent-4-yn-1-yl)carbamate (**1.41**). *tert*-Butyl Benzyl(3-(methoxy(methyl)amino)-3-oxopropyl)carbamate (**1.40**, 10.0 g, 31.1 mmol, 1.00 equiv) was dissolved in dry THF (620 mL) in a 2L round bottom flask under a N<sub>2</sub> atmosphere and cooled to 0 °C. Ethynylmagnesium Bromide (312 mL, 156 mmol, 0.5M in THF, 5.00 equiv) was added dropwise via an addition funnel at 0 °C. Upon completion (~5 h), monitored by TLC, the reaction was quenched with cold 10% HCl. The mixture was extracted with ether (3x), dried with MgSO<sub>4</sub>, and concentrated under reduced pressure. The crude material was purified via SiO<sub>2</sub> flash column chromatography (40% EtOAc/hexanes) to give an orange oil: (8.24 g, 92%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.27 – 1.53 (m, 9H), 2.60 – 2.89 (m, 2H), 3.17 (s, 1H), 3.44 (s, 2H), 4.37 (s, 2H), 7.21 (tdd, *J* = 9.6, 6.6, 5.4 Hz, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 28.4, 41.5, 41.9, 44.3, 44.6, 50.5, 51.6, 79.1, 80.3, 81.3, 127.2, 127.8, 127.3, 128.6, 138.2, 155.4, 185.3; IR (neat) 2977, 2092, 1684, 1415, 1367, 1165 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>17</sub>H<sub>22</sub>NO<sub>3</sub>: 288.1600, found 288.1594.

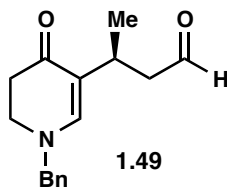


*1-Benzyl-2,3-dihydropyridin-4(1H)-one* (1.42). *tert*-Butyl-benzyl(3-oxopent-4-ynyl)carbamate (1.41, 8.24 g, 28.7 mmol, 1.00 equiv) was dissolved in formic acid (29 mL) in a 100 mL round bottom flask under a N<sub>2</sub> atmosphere to a concentration of 1M. Sodium Iodide (12.9 g, 86.1 mmol, 3.00 equiv) was added and the reaction mixture was allowed to stir for 24h. Passing N<sub>2</sub> over the solution evaporated the formic acid. The formate salt was precipitated with Et<sub>2</sub>O/hexanes (1:1). K<sub>2</sub>CO<sub>3</sub> (19.83g, 143.48 mmol, 5.00 equiv) was slurried in MeOH (1 L) in a 2 L round bottom flask. The salt from step one was dissolved in a minimal amount of MeOH (~50 mL) and added to the K<sub>2</sub>CO<sub>3</sub> slurry slowly over 1.5 h via an addition funnel. Upon completion, monitored via TLC (~1 h), the MeOH was removed via rotary evaporation. The resulting residue was dissolved in H<sub>2</sub>O and was extracted with EtOAc (3x), dried with MgSO<sub>4</sub>, and concentrated under reduced pressure. The crude material was purified via SiO<sub>2</sub> flash column chromatography (100% EtOAc) to give a brown oil: (5.37 g, 71%): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 2.40 – 2.54 (m, 2H), 3.32 – 3.42 (m, 2H), 4.36 (s, 2H), 5.01 (d, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 7.5 Hz, 1H), 7.26 – 7.46 (m, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 35.55, 46.67, 59.82, 98.49, 127.57, 128.28, 128.98, 135.69, 154.10, 191.43; IR (neat) 2897, 1630, 1591, 1361, 1322, 1177 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>12</sub>H<sub>14</sub>NO: 188.1075, found 188.1062.

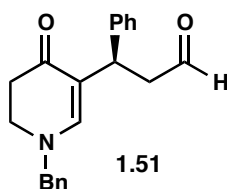


*Representative procedure for organocatalytic reactions:*

Organocatalyst (0.07 mmol, 0.2 equiv) and acid (0.07 mmol, 0.2 equiv) were placed in a capped 1 dram vial and dissolved in an 85:15 mixture of  $\text{CH}_2\text{Cl}_2$  and *i*-PrOH and cooled to  $-78\text{ }^\circ\text{C}$ . The  $\alpha,\beta$ -unsaturated aldehyde (0.98 mmol, 3.00 equiv) was added at the desired temperature and allowed to stir for 5-10 min. The enaminone (0.33 mmol, 1.00 equiv) was dissolved in an 85:15 mixture of  $\text{CH}_2\text{Cl}_2$  and *i*-PrOH and added dropwise to the stirring solution of catalyst, acid, and aldehyde. Upon completion, monitored via TLC (15 min-60 h), the reaction mixture was run through a silica plug and concentrated via rotary evaporation. The crude reaction mixture was purified  $\text{SiO}_2$  flash column chromatography (80% EtOAc/hexanes).

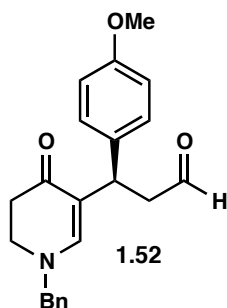


(*S*)-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)butanal (**1.49**). The title compound was obtained as a yellowish oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.15 (d,  $J = 7.1$  Hz, 3H), 2.36 – 2.44 (m, 2H), 2.44 – 2.55 (m, 1H), 2.66 (ddd,  $J = 16.0, 7.2, 2.6$  Hz, 1H), 3.20 (q,  $J = 7.1$  Hz, 1H), 3.27 (t,  $J = 7.9$  Hz, 2H), 4.33 (s, 2H), 7.05 (s, 1H), 7.22 – 7.26 (m, 3H), 7.33-7.41 (m, 3H), 9.69 (t,  $J = 2.4$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  20.29, 26.88, 36.17, 46.73, 50.58, 60.29, 112.79, 127.74, 128.40, 129.14, 136.08, 152.01, 190.01, 203.39; IR (neat) 2962, 2924, 2853, 2727, 1954, 1781, 1674, 1594, 1494, 1452, 1397, 1363, 1322, 1261; HRMS (ESI+)  $m/e$  calc'd  $[\text{M}+\text{H}]^+$  for  $\text{C}_{16}\text{H}_{20}\text{NO}_2^+$ : 258.1489, found 258.1495;  $[\alpha]_{\text{D}}^{23} +33$  ( $c$  1.0,  $\text{CHCl}_3$ ).



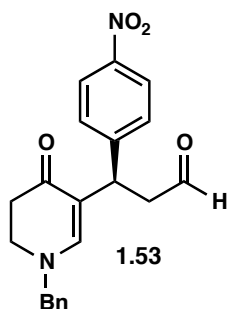
(*R*)-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-phenylpropanal (**1.51**). The title compound was obtained as a yellow oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.38 – 2.49 (m, 2H), 2.90 (ddd,  $J = 16.2, 7.7, 2.3$  Hz, 1H), 3.08 (ddd,  $J = 16.2, 7.9, 2.5$  Hz, 1H), 3.27 (td,  $J = 8.1, 2.0$  Hz, 2H), 4.25 (s, 2H), 4.53 (t,  $J = 7.8$  Hz, 1H), 6.84 (s, 1H), 7.11 – 7.44 (m, 10H), 9.69 (t,  $J = 2.4$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.90, 37.20, 46.63, 48.39, 60.32, 112.03, 126.61, 127.68, 127.93, 128.42, 128.73, 129.12, 135.76, 143.10, 153.52, 189.38, 202.57; IR (neat) 2962, 2924, 2853, 2727, 1954, 1781, 1674, 1594, 1494, 1452, 1397, 1363, 1322, 1261, 1178, 1029, 863, 801, 748, 700  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd  $[\text{M}+\text{H}]^+$  for  $\text{C}_{21}\text{H}_{22}\text{NO}_2^+$ : 320.1645, found 320.1660,  $[\alpha]_{\text{D}}^{23} +1.7$  ( $c$  1.0,  $\text{CHCl}_3$ ).





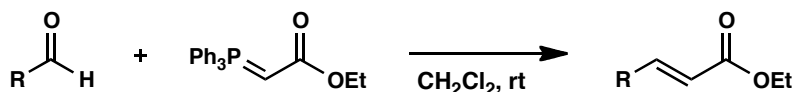
*(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(4-methoxyphenyl)propanal

**(1.52)**. The title compound was isolated as a yellow oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.38 – 2.48 (m, 2H), 2.85 (ddd,  $J = 16.2, 8.0, 2.5$  Hz, 1H), 3.06 (ddd,  $J = 16.2, 7.6, 2.4$  Hz, 1H), 3.22 – 3.31 (m, 2H), 3.79 (s, 3H), 4.25 (s, 2H), 4.47 (t,  $J = 7.8$  Hz, 1H), 6.78 – 6.87 (m, 3H), 7.16 (t,  $J = 6.5$  Hz, 4H), 7.34 (dd,  $J = 9.3, 7.3$  Hz, 3H), 9.67 (t,  $J = 2.2$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.61, 36.20, 46.31, 48.26, 55.07, 59.99, 112.09, 113.78, 127.38, 128.09, 128.64, 128.80, 134.66, 135.50, 153.13, 157.93, 189.14, 202.44; IR (neat) 2918, 2835, 2725, 1720, 1597, 1511, 1453, 1440, 1395, 1364, 1322, 1249, 1179, 1031, 832, 735, 700  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{22}\text{H}_{24}\text{NO}_3^+$ : 350.1751, found 350.1754;  $[\alpha]_{\text{D}}^{23} +4.2$  ( $c$  1.0,  $\text{CHCl}_3$ ).



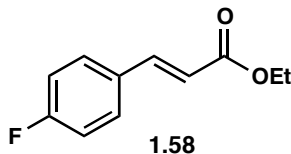
*(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(4-nitrophenyl)propanal (**1.53**).

The title compound was obtained as a yellow oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.38 – 2.46 (m, 2H), 3.02 (ddd,  $J = 17.1, 7.1, 1.5$  Hz, 1H), 3.21 (ddd,  $J = 17.1, 8.1, 2.1$  Hz, 1H), 3.27 – 3.35 (m, 2H), 4.33 (s, 2H), 4.44 (t,  $J = 7.6$  Hz, 1H), 7.00 (s, 1H), 7.15 – 7.22 (m, 2H), 7.32 – 7.39 (m, 3H), 7.42 – 7.51 (m, 2H), 8.07 – 8.18 (m, 2H), 9.71 (t,  $J = 1.7$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.82, 38.02, 46.62, 47.52, 60.31, 110.18, 123.85, 127.72, 128.59, 129.22, 135.44, 146.57, 151.49, 153.31, 189.20, 200.99; 2920, 2820, 1720, 1596, 1395, 1363, 1322, 1186, 749, 699  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{21}\text{H}_{21}\text{N}_2\text{O}_4^+$ : 365.1496, found 365.1501;  $[\alpha]_{\text{D}}^{23} -12$  ( $c$  1.0,  $\text{CHCl}_3$ ).

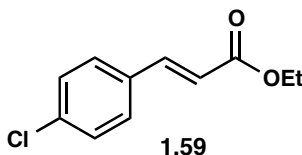


*Representative procedure for the formation of  $\alpha,\beta$ -unsaturated esters:*

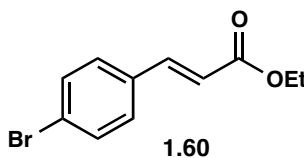
Aldehyde (6.3 mmol, 1.0 equiv) and ethyl (triphenylphosphoranyliden)acetate (7.6 mmol, 1.2 equiv) were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$ . Upon completion, monitored via TLC (12-24 h), the reaction was concentrated. The crude reaction mixture was purified via  $\text{SiO}_2$  flash column chromatography (25-50% EtOAc/hexanes).



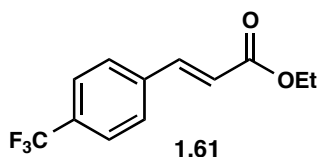
*(E)*-Ethyl 3-(4-Fluorophenyl)acrylate (**1.58**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>338</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.33 (t,  $J = 7.1$  Hz, 3H), 4.26 (q,  $J = 7.1$  Hz, 2H), 6.36 (d,  $J = 16.0$  Hz, 1H), 7.01 – 7.17 (m, 2H), 7.45 – 7.56 (m, 2H), 7.64 (d,  $J = 16.0$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  14.46, 60.70, 116.06, 116.27, 118.15, 118.17, 129.99, 130.08, 130.82, 130.85, 143.41, 162.74, 165.24, 167.02.



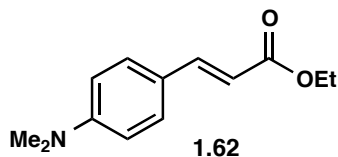
*(E)*-Ethyl 3-(4-Chlorophenyl)acrylate (**1.59**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>338</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.34 (t,  $J = 7.1$  Hz, 3H), 4.27 (q,  $J = 7.1$  Hz, 2H), 6.41 (d,  $J = 16.0$  Hz, 1H), 7.33 – 7.39 (m, 2H), 7.42 – 7.49 (m, 2H), 7.63 (d,  $J = 16.0$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  14.46, 60.78, 119.00, 129.31, 129.35, 133.09, 136.26, 143.28, 166.90.



*(E)*-Ethyl 3-(4-Bromophenyl)acrylate (**1.60**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>339</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.34 (t, *J* = 7.1 Hz, 3H), 4.26 (q, *J* = 7.1 Hz, 2H), 6.42 (d, *J* = 16.0 Hz, 1H), 7.35 – 7.42 (m, 2H), 7.49 – 7.55 (m, 2H), 7.61 (d, *J* = 16.0 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 14.45, 60.79, 119.11, 124.60, 129.57, 132.27, 133.51, 143.34, 166.89.

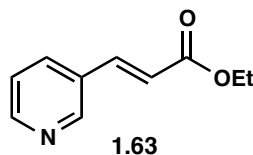


*(E)*-Ethyl 3-(4-(Trifluoromethyl)phenyl)acrylate (**1.61**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>340</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.35 (t, *J* = 7.1 Hz, 3H), 4.28 (q, *J* = 7.1 Hz, 2H), 6.51 (d, *J* = 16.0 Hz, 1H), 7.60 – 7.66 (m, 4H), 7.69 (d, *J* = 16.1 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 166.58, 142.85, 137.96, 131.69, 129.81, 128.30, 126.06, 126.02, 125.99, 125.95, 125.32, 122.61, 120.99, 60.96, 14.43.

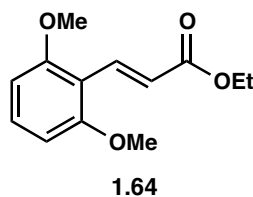


*(E)*-Ethyl 3-(4-(Dimethylamino)phenyl)acrylate (**1.62**). The title compound was obtained as a yellow solid. The spectral data was identical to that reported in the literature.<sup>341</sup> <sup>1</sup>H

NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.32 (t,  $J = 7.1$  Hz, 3H), 3.01 (d,  $J = 4.5$  Hz, 6H), 4.24 (q,  $J = 7.1$  Hz, 2H), 6.22 (d,  $J = 15.8$  Hz, 1H), 6.67 (d,  $J = 8.9$  Hz, 2H), 7.35 – 7.48 (m, 2H), 7.62 (d,  $J = 15.9$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  14.56, 40.29, 60.19, 111.93, 112.72, 122.41, 129.83, 145.22, 151.83, 168.05.

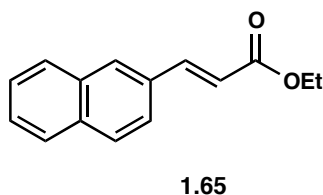


(*E*)-Ethyl 3-(Pyridin-3-yl)acrylate (**1.63**). The title compound was obtained as a yellowish oil. The spectral data was identical to that reported in the literature.<sup>342</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.34 (t,  $J = 7.1$  Hz, 3H), 4.27 (q,  $J = 7.1$  Hz, 2H), 6.50 (d,  $J = 16.1$  Hz, 1H), 7.32 (dd,  $J = 7.9, 4.8$  Hz, 1H), 7.66 (d,  $J = 16.1$  Hz, 1H), 7.75 – 7.90 (m, 1H), 8.59 (dd,  $J = 4.8, 1.5$  Hz, 1H), 8.74 (d,  $J = 2.0$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  14.41, 60.93, 120.59, 123.86, 130.33, 134.30, 140.98, 149.85, 151.11, 166.44.

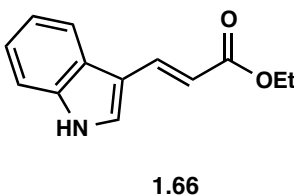


(*E*)-Ethyl 3-(2,6-Dimethoxyphenyl)acrylate (**1.64**). The title compound was obtained as a clear oil. The spectral data was identical to that reported in the literature.<sup>343</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.33 (t,  $J = 7.1$  Hz, 3H), 3.88 (s, 6H), 4.26 (q,  $J = 7.1$  Hz, 2H), 6.56 (d,  $J = 8.4$  Hz, 2H), 6.88 (d,  $J = 16.3$  Hz, 1H), 7.26 (d,  $J = 16.8$  Hz, 1H), 8.13 (d,  $J = 16.3$  Hz,

1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 14.58, 55.90, 60.26, 103.79, 112.46, 120.90, 131.25, 135.51, 160.16, 168.77.

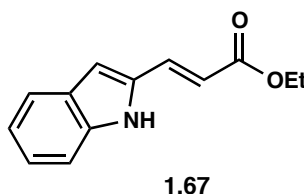


*(E)*-Ethyl 3-(Naphthalen-2-yl)acrylate (**1.65**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>341</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.37 (t, *J* = 7.1 Hz, 3H), 4.30 (q, *J* = 7.1 Hz, 2H), 6.56 (d, *J* = 16.0 Hz, 1H), 7.44 – 7.57 (m, 2H), 7.67 (dd, *J* = 8.6, 1.3 Hz, 1H), 7.78 – 7.89 (m, 4H), 7.93 (s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 14.49, 60.65, 118.59, 123.65, 126.83, 127.33, 127.91, 128.69, 128.81, 130.00, 132.12, 133.43, 134.34, 144.75, 167.19.

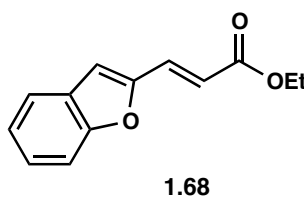


*(E)*-Ethyl 3-(1H-Indol-3-yl)acrylate (**1.66**). The title compound was obtained as a yellow solid. The spectral data was identical to that reported in the literature.<sup>344</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.28 (t, *J* = 7.1 Hz, 3H), 4.21 (q, *J* = 7.1 Hz, 2H), 6.40 (d, *J* = 16.0 Hz, 1H), 7.09 – 7.28 (m, 2H), 7.35 (dd, *J* = 6.8, 1.8 Hz, 1H), 7.41 (d, *J* = 2.7 Hz, 1H), 7.75 – 7.96 (m, 2H), 8.54 (s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 14.58, 60.30, 111.91, 113.62,

113.77, 120.65, 121.64, 123.48, 125.43, 128.93, 137.21, 138.37, 168.51.

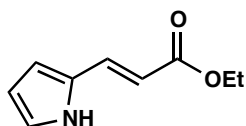


*(E)*-Ethyl 3-(1*H*-Indol-2-yl)acrylate (**1.67**). The title compound was obtained as a yellow solid. The spectral data was identical to that reported in the literature.<sup>345</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.37 (t, *J* = 7.1 Hz, 3H), 4.32 (q, *J* = 7.1 Hz, 2H), 6.32 (d, *J* = 16.0 Hz, 1H), 6.83 (s, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.27 (dd, *J* = 9.4, 5.8 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 16.0 Hz, 1H), 8.77 (s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 14.47, 60.84, 109.03, 111.33, 115.64, 120.70, 121.68, 124.73, 128.52, 133.56, 134.66, 137.98, 167.34.



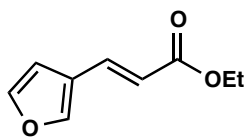
*(E)*-Ethyl 3-(Benzofuran-2-yl)acrylate (**1.68**). The title compound was obtained as a brown solid. The spectral data was identical to that reported in the literature.<sup>346</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.35 (t, *J* = 7.1 Hz, 3H), 4.28 (q, *J* = 7.1 Hz, 2H), 6.58 (dd, *J* = 15.7, 0.4 Hz, 1H), 6.93 (s, 1H), 7.21 – 7.26 (m, 1H), 7.35 (ddd, *J* = 8.4, 7.3, 1.3 Hz, 1H),

7.48 (dd,  $J = 8.3, 0.8$  Hz, 1H), 7.55 (d,  $J = 15.7$  Hz, 1H), 7.58 (ddd,  $J = 7.8, 1.2, 0.7$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  14.44, 60.79, 111.12, 111.54, 119.17, 121.86, 123.44, 126.53, 128.50, 131.34, 152.53, 155.68, 166.82.



**1.69**

*(E)*-Ethyl 3-(1H-Pyrrol-2-yl)acrylate (**1.69**). The title compound was obtained as a yellow solid. The spectral data was identical to that reported in the literature.<sup>347</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.32 (t,  $J = 7.1$  Hz, 3H), 4.24 (q,  $J = 7.1$  Hz, 2H), 6.02 (d,  $J = 15.9$  Hz, 1H), 6.28 (dt,  $J = 3.6, 2.5$  Hz, 1H), 6.47 – 6.63 (m, 1H), 6.93 (td,  $J = 2.7, 1.4$  Hz, 1H), 7.56 (d,  $J = 15.9$  Hz, 1H), 8.80 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  14.5, 60.4, 111.1, 111.4, 114.4, 122.5, 128.6, 134.4, 167.9.

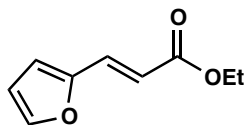


**1.70**

*(E)*-Ethyl 3-(Furan-3-yl)acrylate (**1.70**). The title compound was obtained as a brown oil. The spectral data was identical to that reported in the literature.<sup>348</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.32 (t,  $J = 7.1$  Hz, 3H), 4.24 (q,  $J = 7.1$  Hz, 2H), 6.16 (d,  $J = 15.8$  Hz, 1H), 6.58 (s, 1H), 7.42 (s, 1H), 7.57 (d,  $J = 15.8$  Hz, 1H), 7.64 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz,

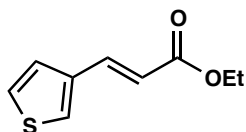


CDCl<sub>3</sub>)  $\delta$  14.46, 60.51, 107.57, 118.18, 122.77, 134.66, 144.52, 144.56, 167.11.



1.71

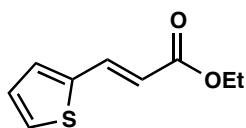
*(E)*-Ethyl 3-(Furan-2-yl)acrylate (**1.71**). The title compound was obtained as a brown solid. The spectral data was identical to that reported in the literature.<sup>340</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.32 (t,  $J$  = 7.1 Hz, 3H), 4.24 (q,  $J$  = 7.1 Hz, 2H), 6.31 (d,  $J$  = 15.7 Hz, 1H), 6.46 (dd,  $J$  = 3.4, 1.8 Hz, 1H), 6.60 (d,  $J$  = 3.4 Hz, 1H), 7.43 (d,  $J$  = 15.7 Hz, 1H), 7.46 – 7.51 (m, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  14.46, 60.59, 112.38, 114.77, 116.09, 131.11, 144.81, 151.09, 167.22.



1.72

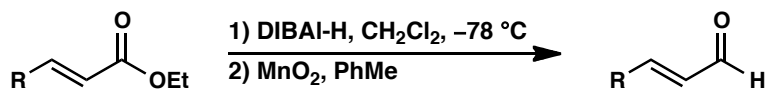
*(E)*-Ethyl 3-(Thiophen-3-yl)acrylate (**1.72**). The title compound was obtained as an orange oil. The spectral data was identical to that reported in the literature.<sup>349</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.33 (t,  $J$  = 7.1 Hz, 3H), 4.25 (q,  $J$  = 7.1 Hz, 2H), 6.26 (d,  $J$  = 15.9 Hz, 1H), 7.28 – 7.31 (m, 1H), 7.34 (ddd,  $J$  = 5.1, 2.9, 0.6 Hz, 1H), 7.49 (dd,  $J$  = 2.9, 1.2 Hz, 1H), 7.60 – 7.75 (m, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  14.48, 60.59, 118.09,

125.29, 127.08, 128.10, 137.75, 138.20, 167.39.



1.73

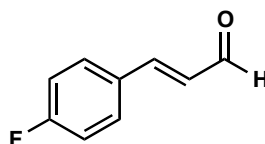
*(E)*-Ethyl 3-(Thiophen-2-yl)acrylate (**1.73**). The title compound was obtained as an orange oil. The spectral data was identical to that reported in the literature.<sup>350</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.33 (t, *J* = 7.1 Hz, 3H), 4.25 (q, *J* = 7.1 Hz, 2H), 6.24 (d, *J* = 15.7 Hz, 1H), 7.05 (dd, *J* = 5.1, 3.6 Hz, 1H), 7.23 – 7.26 (m, 1H), 7.37 (dt, *J* = 5.0, 0.8 Hz, 1H), 7.78 (d, *J* = 15.7 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 14.47, 60.63, 117.21, 128.20, 128.47, 130.94, 137.16, 139.76, 166.99.



*Representative procedure for the synthesis of  $\alpha,\beta$ -unsaturated aldehydes:*

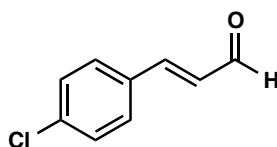
The  $\alpha,\beta$ -unsaturated ester (5.0 mmol, 1.0 equiv) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> to a concentration of 0.167M and cooled to -78 °C. DIBAL-H (10.5 mmol, 2.10 equiv, 1.0 M solution in toluene) was added dropwise. Upon completion (monitored via TLC, 15-45 min) the reaction was carefully quenched with 10% aqueous NaOH at -78 °C and warmed to rt. The organic phase was separated and the aqueous phase was further

washed with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were dried with MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The crude mixture was then dissolved in toluene to a concentration of 0.65M and MnO<sub>2</sub> (22.5 mmol, 4.50 equiv). Upon completion (monitored via TLC, 24-48 h) the solids were filtered off and the filtrate was concentrated to give the desired  $\alpha,\beta$ -unsaturated aldehyde. The aldehydes were used in the organocatalytic reaction without further purification.



1.74

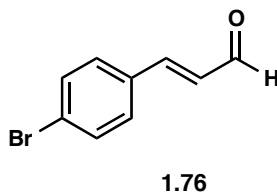
*(E)*-3-(4-Fluorophenyl)acrylaldehyde (**1.74**). The title compound was isolated as a colorless oil. The spectral data was identical to that reported in the literature.<sup>351</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.63 (dd,  $J$  = 15.9, 7.6 Hz, 1H), 7.14-7.10 (m, 2H), 7.44 (d,  $J$  = 15.9 Hz, 1H), 7.58-7.55 (m, 2H), 9.68 (d,  $J$  = 7.6 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  116.3, 128.3, 130.3, 130.5, 151.3, 164.4, 193.4.



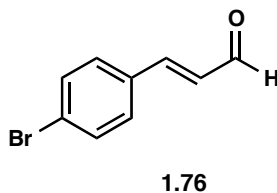
1.75

*(E)*-3-(4-Chlorophenyl)acrylaldehyde (**1.75**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>351</sup> <sup>1</sup>H NMR (400

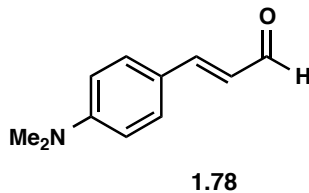
MHz, CDCl<sub>3</sub>)  $\delta$  6.69 (dd,  $J = 16.0, 7.6$  Hz, 1H), 7.54-7.39 (m, 5H), 9.71 (d,  $J = 7.6$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  128.9, 129.4, 129.6, 132.5, 137.2, 151.3, 193.4.



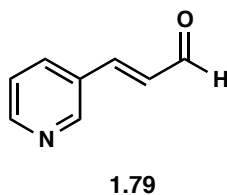
*(E)*-3-(4-Bromophenyl)acrylaldehyde (**1.76**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>351</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.62 (dd,  $J = 16.0, 7.6$  Hz, 1H), 7.30 – 7.37 (m, 3H), 7.46 – 7.52 (m, 2H), 9.63 (d,  $J = 7.6$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  126.0, 127.8, 128.3, 131.2, 152.7, 155.0, 193.7.



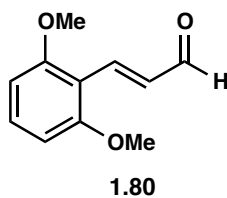
*(E)*-3-(4-(Trifluoromethyl)phenyl)acrylaldehyde (**1.77**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>352</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.65 (dd,  $J = 16.1, 7.5$  Hz, 1H), 7.39 (d,  $J = 16.0$  Hz, 1H), 7.55 (d,  $J = 1.0$  Hz, 4H), 9.63 (dd,  $J = 7.5, 0.8$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  126.2, 126.3, 128.8, 130.9, 150.9, 193.1.



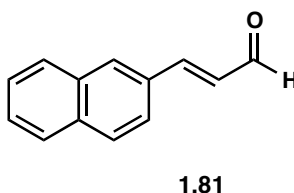
*(E)*-3-(4-(Dimethylamino)phenyl)acrylaldehyde (**1.78**). The title compound was obtained as a yellow solid. The spectral data was identical to that reported in the literature.<sup>351</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.05 (s, 6H), 6.55 (dd, *J* = 15.6, 7.9 Hz, 1H), 6.67 – 6.70 (m, 2H), 7.38 (d, *J* = 15.6 Hz, 1H), 7.41 – 7.51 (m, 2H), 9.59 (d, *J* = 7.9 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 40.22, 111.90, 123.99, 127.63, 130.64, 152.54, 154.03, 193.87.



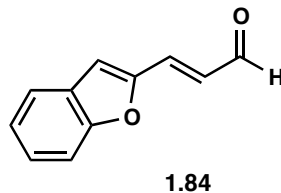
*(E)*-3-(Pyridin-3-yl)acrylaldehyde (**1.79**). The title compound was obtained as a yellow solid. The spectral data was identical to that reported in the literature.<sup>353</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.77 (dd, *J* = 16.1, 7.5 Hz, 1H), 7.38 (dd, *J* = 7.9, 4.9 Hz, 1H), 7.48 (d, *J* = 16.1 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 8.65 (dd, *J* = 4.1, 0.6 Hz, 1H), 8.78 (d, *J* = 1.9 Hz, 1H), 9.74 (d, *J* = 7.5 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 124.06, 129.92, 130.34, 134.51, 148.58, 150.21, 152.02, 193.09.



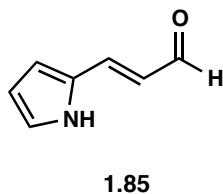
*(E)*-3-(2,6-Dimethoxyphenyl)acrylaldehyde (**1.80**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>353</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.89 (s, 6H), 6.57 (d, *J* = 8.4 Hz, 2H), 7.16 (dd, *J* = 16.1, 8.1 Hz, 1H), 7.32 (t, *J* = 8.4 Hz, 1H), 7.92 (d, *J* = 16.1 Hz, 1H), 9.63 (d, *J* = 8.1 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 56.22, 104.10, 112.50, 132.08, 133.04, 144.95, 160.45, 196.94.



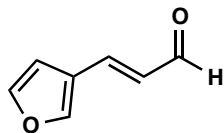
*(E)*-3-(Naphthalen-2-yl)acrylaldehyde (**1.81**). The title compound was obtained as a white solid. The spectral data was identical to that reported in the literature.<sup>339</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.83 (dd, *J* = 15.9, 7.7 Hz, 1H), 7.55 (ddd, *J* = 5.0, 2.6, 1.8 Hz, 2H), 7.62 (d, *J* = 15.9 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.87 (dd, *J* = 15.2, 6.7 Hz, 3H), 7.98 (s, 1H), 9.76 (d, *J* = 7.7 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 123.5, 126.9, 127.7, 127.8, 128.6, 128.7, 128.9, 130.6, 131.5, 133.1, 134.6, 152.6, 193.5.



*(E)*-3-(Benzofuran-2-yl)acrylaldehyde (**1.84**). The title compound was obtained as an orange solid. The spectral data was identical to that reported in the literature.<sup>354</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.82 (dd, *J* = 15.6, 7.8 Hz, 1H), 7.09 (s, 1H), 7.25–7.29 (m, 1H), 7.35 (d, *J* = 15.6 Hz, 1H), 7.39–7.43 (m, 1H), 7.50–7.53 (m, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 9.72 (d, *J* = 7.8 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 111.7, 112.9, 122.0, 123.6, 127.2, 128.2, 128.4, 137.8, 151.9, 155.9, 192.7.

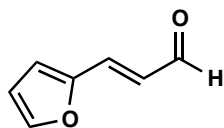


*(E)*-3-(1H-Pyrrol-2-yl)acrylaldehyde (**1.85**). The title compound was obtained as a yellow oil. The spectral data was identical to that reported in the literature.<sup>355</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.39 – 6.31 (m, 1H), 6.45 (dd, *J* = 15.7, 7.9 Hz, 1H), 6.68 (d, *J* = 3.6 Hz, 1H), 7.07 (d, *J* = 1.0 Hz, 1H), 7.34 (d, *J* = 15.7 Hz, 1H), 9.48 (s, 1H), 9.54 (d, *J* = 7.9 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 111.8, 117.7, 121.9, 124.9, 128.6, 142.5, 193.7.



**1.86**

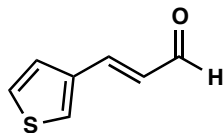
*(E)*-3-(Furan-3-yl)acrylaldehyde (**1.86**). The title compound was obtained as an orange oil. The spectral properties were identical to that reported in the literature.<sup>356</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.43 (dd,  $J = 15.8, 7.8$  Hz, 1H), 6.61 (dd,  $J = 1.3, 0.6$  Hz, 1H), 7.39 (d,  $J = 15.8$  Hz, 1H), 7.47 (d,  $J = 1.1$  Hz, 1H), 7.74 (s, 1H), 9.62 (d,  $J = 7.8$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  107.9, 123.2, 129.2, 142.8, 145.3, 145.7, 193.8.



**1.87**

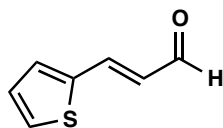
*(E)*-3-(Furan-2-yl)acrylaldehyde (**1.87**). The title compound was obtained as an orange oil. The spectral data was identical to that reported in the literature.<sup>356</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.52 (dd,  $J = 3.5, 1.8$  Hz, 1H), 6.57 (dd,  $J = 15.7, 7.9$  Hz, 1H), 6.76 (d,  $J = 3.4$  Hz, 1H), 7.21 (d,  $J = 15.7$  Hz, 1H), 7.55 (d,  $J = 1.6$  Hz, 1H), 9.61 (d,  $J = 7.9$  Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  112.98, 116.81, 126.14, 137.90, 146.02, 150.72, 192.98.





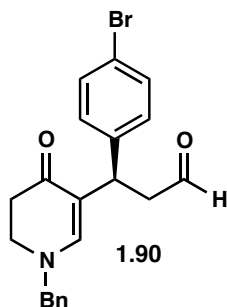
1.88

*(E)*-3-(Thiophen-3-yl)acrylaldehyde (**1.88**). The title compound was obtained as an orange oil. The spectral data was identical to that reported in the literature.<sup>353</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.53 (dd, *J* = 15.8, 7.8 Hz, 1H), 7.32 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.39 (dd, *J* = 5.1, 2.9 Hz, 1H), 7.47 (d, *J* = 15.8 Hz, 1H), 7.62 (dd, *J* = 2.8, 0.9 Hz, 1H), 9.65 (d, *J* = 7.8 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 125.47, 127.59, 128.55, 129.70, 137.53, 145.86, 193.91.



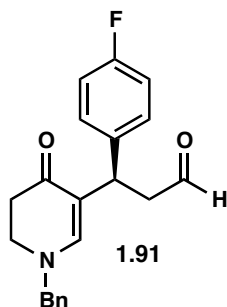
1.89

*(E)*-3-(Thiophen-2-yl)acrylaldehyde (**1.89**). The title compound was obtained as an orange oil. The spectral data was identical to that reported in the literature.<sup>357</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.51 (dd, *J* = 15.6, 7.7 Hz, 1H), 7.11 (dd, *J* = 5.0, 3.7 Hz, 1H), 7.33 – 7.38 (m, 1H), 7.50 (d, *J* = 5.0 Hz, 1H), 7.58 (d, *J* = 15.6 Hz, 1H), 9.62 (d, *J* = 7.7 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 127.50, 128.65, 130.50, 132.18, 139.40, 144.50, 192.98.



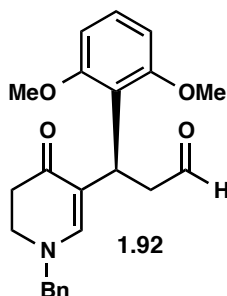
*(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(4-bromophenyl)propanal

**(1.90)**. The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.42 (t,  $J = 7.8$  Hz, 2H), 2.89 (ddd,  $J = 16.5, 7.6, 1.7$  Hz, 1H), 3.09 (ddd,  $J = 16.5, 7.9, 2.1$  Hz, 1H), 3.28 (dd,  $J = 11.9, 4.6$  Hz, 2H), 4.28 (s, 2H), 4.41 (t,  $J = 7.8$  Hz, 1H), 6.86 (s, 1H), 7.15 (t,  $J = 7.6$  Hz, 4H), 7.28 – 7.47 (m, 5H), 9.68 (t,  $J = 1.9$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.88, 37.07, 46.65, 48.06, 60.28, 111.38, 120.33, 127.71, 128.49, 129.16, 129.62, 131.71, 135.64, 142.42, 153.25, 189.28, 201.89; IR (neat) 2898, 2839, 2725, 1721, 1631, 1598, 1488, 1453, 1435, 1394, 1363, 1322, 1282, 1205, 1187, 1074, 1009, 821, 734, 700  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{21}\text{H}_{21}\text{BrNO}_2^+$ : 398.0750, found 398.0680;  $[\alpha]_{\text{D}}^{23} +9.9$  ( $c$  1.0,  $\text{CHCl}_3$ ).



*(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(4-fluorophenyl)propanal (**1.91**).

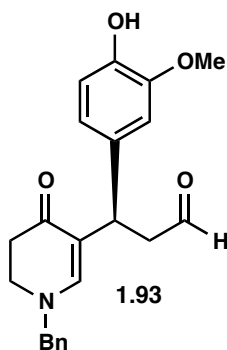
The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.32 – 2.51 (m, 2H), 2.89 (ddd,  $J = 16.4, 7.7, 2.1$  Hz, 1H), 3.09 (ddd,  $J = 16.4, 7.9, 2.3$  Hz, 1H), 3.28 (td,  $J = 8.2, 1.4$  Hz, 2H), 4.27 (s, 2H), 4.45 (t,  $J = 7.8$  Hz, 1H), 6.85 (s, 1H), 6.93 – 7.02 (m, 2H), 7.13 – 7.20 (m, 2H), 7.20 – 7.25 (m, 2H), 7.31 – 7.40 (m, 3H), 9.68 (t,  $J = 2.2$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.89, 36.85, 46.67, 48.41, 60.32, 111.81, 115.36, 115.57, 127.72, 128.49, 129.17, 129.30, 129.38, 135.68, 138.96, 153.35, 189.41, 202.16; IR (neat) 2918, 2835, 1720, 1595, 1508, 1453, 1395, 1322, 1186, 836, 699  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{21}\text{H}_{21}\text{FNO}_2^+$ : 338.1551, found 338.1562;  $[\alpha]_{\text{D}}^{23}$   $-15$  (c 1.0,  $\text{CHCl}_3$ ).



*(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(2,6-dimethoxyphenyl) propanal

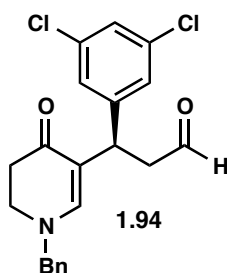
**(1.92)**. The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.45 (dt,  $J = 14.4, 6.6$  Hz, 2H), 2.91 (ddd,  $J = 16.2, 8.6, 2.7$  Hz, 1H), 2.98 (ddd,  $J = 16.1, 6.6, 2.9$  Hz, 1H), 3.20 – 3.35 (m, 2H), 3.77 (s, 6H), 4.23 (d,  $J = 5.9$  Hz, 2H), 5.25 (dd,  $J = 8.5, 6.7$  Hz, 1H), 6.54 (d,  $J = 8.3$  Hz, 2H), 6.91 (s, 1H), 7.13 – 7.21 (m, 3H), 7.28 – 7.38 (m, 3H),

9.62 (t,  $J = 2.8$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  27.08, 35.70, 46.71, 46.76, 55.47, 59.97, 104.37, 109.59, 117.94, 127.39, 127.85, 127.91, 128.69, 135.91, 153.34, 158.36, 189.37, 204.04; IR (neat) 2918, 2835, 2725, 1720, 1597, 1511, 1453, 1440, 1395, 1364, 1322, 1249, 1179, 1031, 832, 735,  $700\text{ cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{23}\text{H}_{26}\text{NO}_4^+$ : 380.1856, found 380.1854;  $[\alpha]_{\text{D}}^{23} +7.2$  ( $c$  1.0,  $\text{CHCl}_3$ ).

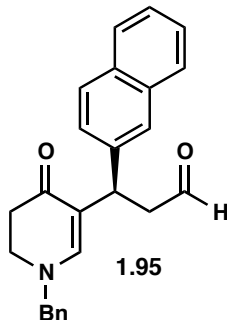


*(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(4-hydroxy-3-methoxyphenyl)propanal (**1.93**). The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.37 – 2.50 (m, 2H), 2.84 (ddd,  $J = 16.2, 8.2, 2.6$  Hz, 1H), 3.03 (ddd,  $J = 16.2, 7.6, 2.4$  Hz, 1H), 3.27 (td,  $J = 8.0, 2.3$  Hz, 2H), 3.85 (s, 3H), 4.25 (s, 2H), 4.45 (t,  $J = 7.8$  Hz, 1H), 5.67 (s, 1H), 6.70 (dd,  $J = 8.1, 2.0$  Hz, 1H), 6.75 – 6.90 (m, 3H), 7.14 (dd,  $J = 7.7, 1.8$  Hz, 2H), 7.27 – 7.42 (m, 3H), 9.67 (t,  $J = 2.4$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.87, 36.96, 46.68, 48.60, 56.04, 60.27, 111.08, 112.40, 114.40, 120.04, 127.69, 128.40, 129.09, 134.92, 135.74, 144.32, 146.75, 153.50, 189.41, 202.64; IR

(neat) 2924, 2821, 2725, 1710, 1597, 1536, 1459, 1431, 1360, 1336, 1322, 1269, 1163, 1031, 832, 735, 700  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{22}\text{H}_{24}\text{NO}_4^+$ : 366.1700, found 366.1714;  $[\alpha]_{\text{D}}^{23} +13$  ( $c$  1.0,  $\text{CHCl}_3$ ).

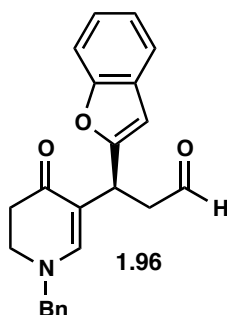


*(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(3,5-dichlorophenyl)propanal  
**(1.94)**. The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.31 – 2.53 (m, 2H), 2.92 (ddd,  $J = 16.9, 7.1, 1.4$  Hz, 1H), 3.09 (ddd,  $J = 16.9, 8.3, 2.2$  Hz, 1H), 3.30 (t,  $J = 7.9, 7.9$  Hz, 2H), 4.24 – 4.43 (m, 3H), 6.96 (s, 1H), 7.13 – 7.23 (m, 5H), 7.30 – 7.43 (m, 3H), 9.68 (t,  $J = 1.7, 1.7$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.75, 37.29, 46.59, 47.65, 60.31, 110.28, 126.34, 126.71, 127.71, 128.54, 129.21, 134.98, 135.48, 147.25, 153.30, 189.15, 201.12; IR (neat) 2918, 2836, 1721, 1596, 1433, 1395, 1363, 1323, 1187, 797, 699  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{21}\text{H}_{20}\text{Cl}_2\text{NO}_2^+$ : 388.0866, found 388.0881;  $[\alpha]_{\text{D}}^{23} +8.1$  ( $c$  1.0,  $\text{CHCl}_3$ ).



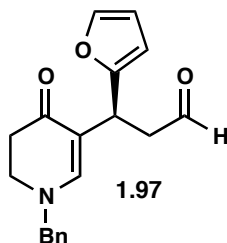
*(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(naphthalen-2-yl)propanal

(1.95). The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.42 – 2.50 (m, 2H), 3.02 (ddd,  $J = 16.2, 7.9, 2.3$  Hz, 1H), 3.15 (ddd,  $J = 16.2, 7.7, 2.5$  Hz, 1H), 3.28 (td,  $J = 8.1, 2.6$  Hz, 2H), 4.22 (s, 2H), 4.73 (t,  $J = 7.8$  Hz, 1H), 6.83 (s, 1H), 7.07 – 7.17 (m, 2H), 7.28 – 7.36 (m, 3H), 7.38 (dd,  $J = 8.5, 1.7$  Hz, 1H), 7.41 – 7.50 (m, 2H), 7.66 (s, 1H), 7.79 (t,  $J = 7.0$  Hz, 3H), 9.74 (t,  $J = 2.4$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.90, 37.08, 46.69, 48.32, 60.29, 112.05, 125.78, 126.25, 127.07, 127.69, 127.94, 128.41, 128.48, 129.10, 132.42, 133.59, 135.71, 140.63, 153.67, 189.32, 202.51; IR (neat) 2920, 2820, 1720, 1596, 1395, 1363, 1322, 1186, 749, 699  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{25}\text{H}_{24}\text{NO}_2^+$ : 370.1802, found 370.1801;  $[\alpha]_{\text{D}}^{23} +17$  ( $c$  1.0,  $\text{CHCl}_3$ ).

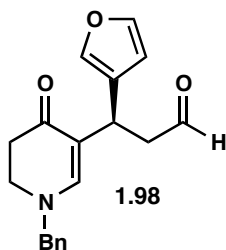


*(S)*-3-(Benzofuran-2-yl)-3-(1-benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)propanal

**(1.96).** The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.48 (ddd,  $J = 7.4, 5.3, 1.8$  Hz, 2H), 3.02 (dd,  $J = 7.5, 2.2$  Hz, 2H), 3.33 (t,  $J = 7.9$  Hz, 2H), 4.30 (s, 2H), 4.75 (t,  $J = 7.4$  Hz, 1H), 6.49 (s, 1H), 7.09 (s, 1H), 7.16 – 7.27 (m, 5H), 7.28 – 7.38 (m, 3H), 7.38 – 7.44 (m, 1H), 7.44 – 7.54 (m, 1H), 9.76 (t,  $J = 2.1$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  31.47, 35.68, 46.68, 47.12, 60.35, 103.39, 108.53, 111.05, 120.81, 122.81, 123.76, 127.71, 128.48, 128.68, 129.14, 135.60, 153.47, 154.86, 159.07, 188.79, 201.53; IR (neat) 2920, 2880, 2727, 1722, 1596, 1454, 1396, 1323, 1187, 1080, 942, 808, 752, 700  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{23}\text{H}_{23}\text{NO}_3^+$ : 360.1594, found 360.1615;  $[\alpha]_{\text{D}}^{23} +13$  ( $c$  1.0,  $\text{CHCl}_3$ ).



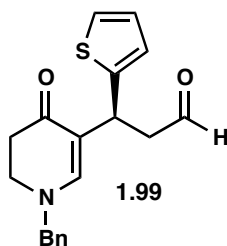
(*S*)-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(furan-2-yl)propanal (**1.97**). The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.38 – 2.54 (m, 2H), 2.81 – 2.96 (m, 2H), 3.30 (t,  $J = 7.9$  Hz, 2H), 4.30 (s, 2H), 4.61 (t,  $J = 7.4$  Hz, 1H), 6.08 (d,  $J = 3.2$  Hz, 1H), 6.29 (dd,  $J = 3.2, 1.9$  Hz, 1H), 6.95 (s, 1H), 7.16 – 7.22 (m, 2H), 7.28 – 7.41 (m, 4H), 9.70 (t,  $J = 2.3$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  29.95, 34.54, 45.49, 46.30, 59.20, 105.21, 108.21, 109.25, 126.54, 127.29, 127.99, 134.60, 140.51, 152.22, 154.77, 187.73, 200.83; IR (neat) 2960, 2901, 2842, 2727, 1721, 1596, 1504, 1488, 1397, 1362, 1323, 1276, 1187, 1144, 1081, 1009, 929, 807, 736, 701  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{19}\text{H}_{20}\text{NO}_3^+$ : 310.1438, found 310.1454;  $[\alpha]_{\text{D}}^{23} +6.9$  ( $c$  1.0,  $\text{CHCl}_3$ ).



(*R*)-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(furan-3-yl)propanal (**1.98**). The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.40 – 2.51 (m, 2H), 2.76 (ddd,  $J = 16.1, 8.0, 2.6$  Hz, 1H), 2.91 (ddd,  $J = 16.1, 7.0, 2.3$  Hz, 1H), 3.30 (dd,  $J = 12.0, 4.4$  Hz, 2H), 4.28 (s, 2H), 4.41 (t,  $J = 7.5$  Hz, 1H), 6.26 (dd,  $J = 1.6, 0.8$  Hz, 1H), 6.93 (s,



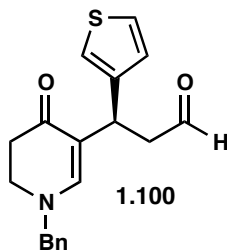
1H), 7.14 – 7.21 (m, 2H), 7.23 – 7.26 (m, 1H), 7.30 – 7.42 (m, 4H), 9.70 (t,  $J = 2.4$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  28.66, 35.84, 46.68, 48.77, 60.30, 110.67, 110.97, 127.11, 127.68, 128.45, 129.14, 135.79, 139.55, 143.39, 153.41, 189.26, 202.53; ; IR (neat) 2960, 2901, 2842, 2727, 1721, 1596, 1504, 1488, 1397, 1362, 1323, 1276, 1187, 1144, 1081, 1009, 929, 807, 736, 701  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{19}\text{H}_{20}\text{NO}_3^+$ : 310.1438, found 310.1450;  $[\alpha]_{\text{D}}^{23} +7.4$  ( $c$  1.0,  $\text{CHCl}_3$ ).



*(S)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(thiophen-2-yl)propanal (**1.99**).

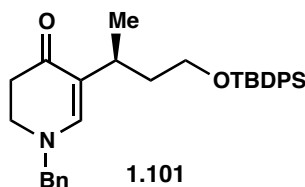
The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow orange oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.45 (td,  $J = 7.6, 2.5$  Hz, 2H), 2.96 (ddd,  $J = 16.4, 7.6, 1.9$  Hz, 1H), 3.07 (ddd,  $J = 16.4, 7.5, 2.1$  Hz, 1H), 3.30 (t,  $J = 7.8$  Hz, 2H), 4.30 (s, 2H), 4.77 (t,  $J = 7.6$  Hz, 1H), 6.86 (d,  $J = 3.2$  Hz, 1H), 6.89 – 6.95 (m, 1H), 7.00 (s, 1H), 7.17 (dd,  $J = 15.3, 6.0$  Hz, 3H), 7.29 – 7.42 (m, 3H), 9.71 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  32.93, 35.75, 46.58, 49.64, 60.35, 111.60, 124.00, 124.44, 126.89, 127.69, 128.45, 129.15, 135.69, 147.42, 153.52, 188.83, 201.88; IR (neat) 2920, 2848, 1720, 1596, 1453, 1396, 1362, 1323, 1187, 1080, 1028,

850, 699  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{19}\text{H}_{20}\text{NO}_2\text{S}^+$ : 326.1209, found 326.1212;  $[\alpha]_{\text{D}}^{23} +6.1$  ( $c$  1.0,  $\text{CHCl}_3$ ).



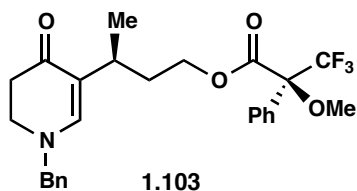
*(S)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-(thiophen-3-yl)propanal (**1.100**).

The title compound was prepared via the general organocatalysis procedure described earlier and isolated as a yellow orange oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.27 – 2.54 (m, 2H), 2.80 (ddd,  $J = 16.1, 7.9, 2.5$  Hz, 1H), 2.92 (ddd,  $J = 16.1, 7.3, 2.4$  Hz, 1H), 3.12 – 3.29 (m, 2H), 4.18 (s, 2H), 4.53 (t,  $J = 7.6$  Hz, 1H), 6.74 (s, 1H), 6.87 (dd,  $J = 5.0, 1.3$  Hz, 1H), 6.90 – 6.94 (m, 1H), 7.05 – 7.10 (m, 2H), 7.19 (dd,  $J = 4.8, 3.0$  Hz, 1H), 7.22 – 7.32 (m, 3H), 9.61 (t,  $J = 2.4$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  32.87, 35.79, 46.62, 48.89, 60.22, 111.45, 120.73, 126.00, 127.63, 127.95, 128.37, 129.06, 135.69, 143.89, 153.46, 189.10, 202.50; IR (neat) 2897, 2839, 2726, 1720, 1596, 1453, 1396, 1322, 1187, 1080, 839, 772, 736, 700  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{19}\text{H}_{20}\text{NO}_2\text{S}^+$ : 326.1209, found 326.1219;  $[\alpha]_{\text{D}}^{23} +5.6$  ( $c$  1.0,  $\text{CHCl}_3$ ).



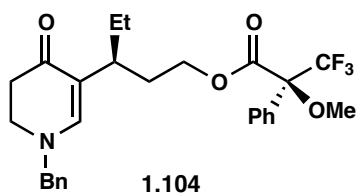
(*S*)-1-Benzyl-5-(4-((*tert*-butyldiphenylsilyl)oxy)butan-2-yl)-2,3-dihydropyridin-4(1*H*)-one (**1.101**). (*S*)-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)butanal (**1.49**, 0.158 g, 0.6 mmol, 1.00 equiv) was dissolved in MeOH and cooled to  $-15\text{ }^{\circ}\text{C}$ . Sodium borohydride (0.023 g, 0.60 mmol, 1.00 equiv) was added in one portion and the mixture was allowed to stir for 20 minutes. The mixture was concentrated under reduced pressure and the crude mixture was taken up in  $\text{CH}_2\text{Cl}_2$ , filtered, and concentrated under reduced pressure to yield the crude alcohol, which was used in the next step without purification. The alcohol was dissolved in  $\text{CH}_2\text{Cl}_2$  and cooled to  $0\text{ }^{\circ}\text{C}$  when *tert*-butyldiphenylsilyl chloride (0.203 g, 0.74 mmol, 1.2 equiv) and imidazole (0.051 g, 0.74 mmol, 1.2 equiv) and allowed to stir until completion (3 h). The reaction mixture was quenched with water and extracted with  $\text{CH}_2\text{Cl}_2$ . The combined organic phases were dried over  $\text{MgSO}_4$ , concentrated under reduced pressure and purified by  $\text{SiO}_2$  flash chromatography (25% EtOAc/hexanes) to give 0.12 g (39%) of the title compound as a clear oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.05 (m, 12H), 1.69 (dq,  $J = 13.7, 7.0\text{ Hz}$ , 1H), 1.75 – 1.92 (m, 1H), 2.38 (dd,  $J = 8.4, 7.2\text{ Hz}$ , 2H), 2.75 (q,  $J = 7.1\text{ Hz}$ , 1H), 3.20 (dd,  $J = 8.4, 7.2\text{ Hz}$ , 2H), 3.55 – 3.75 (m, 2H), 4.23 (s, 2H), 6.91 (s, 1H), 7.21 (dd,  $J = 7.6, 1.8\text{ Hz}$ , 2H), 7.29 – 7.44 (m, 9H), 7.65 – 7.71 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  19.35, 21.15, 27.03, 28.43,

36.45, 38.95, 46.81, 60.14, 62.90, 114.66, 127.68, 127.69, 128.20, 129.02, 129.58, 134.31, 134.34, 135.71, 136.42, 151.64, 190.22; IR (neat) 2920, 2820, 1720, 1596, 1395, 1363, 1322, 1186, 749, 699  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{32}\text{H}_{40}\text{NO}_2\text{Si}^+$ : 498.2823, found 498.2801.



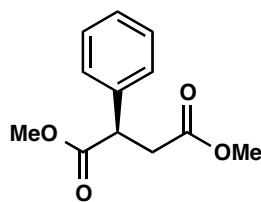
*(S)*-*(S)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)butyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (**1.103**). *(S)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)butanal (**1.49**, 0.038 g, 0.15 mmol, 1.00 equiv) was dissolved in MeOH and cooled to  $-15\text{ }^{\circ}\text{C}$ . Sodium borohydride (0.006 g, 0.15 mmol 1.00 quiv) was added in one portion and the mixture was allowed to stir for 20 minutes. The mixture was concentrated under reduced pressure and the crude mixture was taken up in  $\text{CH}_2\text{Cl}_2$ , filtered, and concentrated under reduced pressure to yield the crude alcohol, which was used in the next step without purification. The crude alcohol and DMAP (0.009 g, 0.08 mmol, 0.5 equiv) was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$ . *(S)*-MTPA-Cl (0.072 g, 0.29 mmol, 1.9 equiv) was added dropwise and the reaction progress was monitored via TLC. Upon completion (1 h), the reaction mixture was quenched with water and the organic layer was separated and the aqueous layer was back extracted with  $\text{CH}_2\text{Cl}_2$  (3x). The combined organic extracts were dried ( $\text{MgSO}_4$ ), filtered, concentrated under reduced pressure, and

purified via SiO<sub>2</sub> flash chromatography (25 % EtOAc/hexanes) to yield 0.067 g (93%) of the title compound as a clear oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.11 (d, *J* = 6.9 Hz, 3H), 1.81 (ddd, *J* = 13.8, 6.4, 1.4 Hz, 1H), 1.88 – 2.02 (m, 1H), 2.50 (ddd, *J* = 9.9, 7.5, 2.2 Hz, 2H), 2.63 (dt, *J* = 8.3, 6.6 Hz, 1H), 3.29 (t, *J* = 8.0 Hz, 2H), 3.55 (s, 3H), 4.26 – 4.37 (m, 4H), 7.03 (s, 1H), 7.20 – 7.25 (m, 2H), 7.38 (dd, *J* = 5.7, 2.1 Hz, 4H), 7.52 (dd, *J* = 6.7, 3.1 Hz, 2H), 7.61 (dd, *J* = 6.8, 3.0 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 14.18, 20.37, 21.04, 28.68, 28.97, 34.54, 34.66, 35.08, 35.13, 46.29, 55.31, 55.40, 60.18, 60.20, 60.47, 65.45, 65.49, 112.28, 127.38, 127.50, 127.56, 128.27, 128.37, 128.40, 128.43, 129.06, 129.32, 129.59, 132.67, 135.55, 135.57, 153.34, 153.38, 166.59, 190.59, 190.61; <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>) δ -71.61, -71.47; IR (neat) 2962, 2924, 2853, 2727, 1954, 1781, 1739, 1674, 1594, 1494, 1452, 1397, 1363, 1322, 1261; HRMS (ESI+) *m/e* calc'd [M+H]<sup>+</sup> for C<sub>26</sub>H<sub>29</sub>F<sub>3</sub>NO<sub>4</sub><sup>+</sup>: 476.2043, found 476.2050; [α]<sub>D</sub><sup>23</sup> -46 (*c* 1.0, CHCl<sub>3</sub>).



(*S*)-(*S*)-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)pentyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (**1.104**). (*S*)-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)butanal (**1.49**, 0.049 g, 0.19 mmol, 1.00 equiv) was dissolved in MeOH and cooled to -15 °C. Sodium borohydride (0.007 g, 0.2 mmol 1.0 quiv) was added in one portion and the mixture was allowed to stir for 20 minutes. The mixture was concentrated under

reduced pressure and the crude mixture was taken up in CH<sub>2</sub>Cl<sub>2</sub>, filtered, and concentrated under reduced pressure to yield the crude alcohol, which was used in the next step without purification. The crude alcohol and DMAP (0.012 g, 0.077 mmol, 0.50 equiv) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub>. (*S*)-MTPA-Cl (0.091 g, 0.36 mmol, 1.9 equiv) was added dropwise and the reaction progress was monitored via TLC. Upon completion (1 h), the reaction mixture was quenched with water and the organic layer was separated and the aqueous layer was back extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x). The combined organic extracts were dried (MgSO<sub>4</sub>), filtered, concentrated under reduced pressure, and purified via SiO<sub>2</sub> flash chromatography (25 % EtOAc/hexanes) to yield 0.067 g (72%) of the title compound as a clear oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 0.74 – 0.86 (m, 3H), 1.48 (dddd, *J* = 13.3, 7.2, 5.6, 2.3 Hz, 1H), 1.58 (ddd, *J* = 13.6, 9.0, 7.3 Hz, 1H), 1.83 – 2.03 (m, 2H), 2.21 (tt, *J* = 10.0, 5.1 Hz, 1H), 2.41 (t, *J* = 7.8 Hz, 2H), 3.26 (t, *J* = 7.8 Hz, 2H), 3.54 (d, *J* = 1.3 Hz, 3H), 4.18 – 4.41 (m, 4H), 6.87 (s, 1H), 7.21 – 7.25 (m, 2H), 7.31 – 7.42 (m, 6H), 7.53 (dt, *J* = 7.7, 3.4 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 12.32, 12.35, 27.15, 27.20, 32.67, 32.76, 36.33, 36.38, 37.57, 46.70, 46.72, 55.46, 55.47, 60.09, 60.11, 65.67, 65.69, 110.03, 127.45, 127.49, 127.50, 127.61, 128.27, 128.48, 128.51, 129.06, 129.64, 129.66, 132.42, 136.10, 152.88, 152.96, 166.63, 190.36, 190.38; <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>) δ -71.47, -71.61; IR (neat) 2962, 2924, 2853, 2727, 1954, 1781, 1739, 1674, 1594, 1494, 1452, 1397, 1363, 1322, 1261; HRMS (ESI+) *m/e* calc'd [M+H]<sup>+</sup> for C<sub>27</sub>H<sub>31</sub>F<sub>3</sub>NO<sub>4</sub><sup>+</sup>: 490.2200, found 476.2191; [α]<sub>D</sub><sup>23</sup> -47 (*c* 1.0, CHCl<sub>3</sub>).

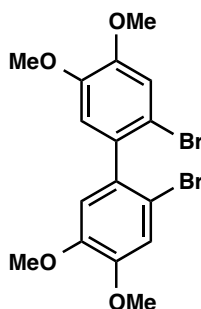


1.105

*(R)*-Dimethyl-2-phenylsuccinate (1.105). *(R)*-3-(1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)-3-phenylpropanal (0.092 g, 0.3 mmol, 1.00 equiv) was placed in a 2 dram vial, dissolved in CH<sub>2</sub>Cl<sub>2</sub>, and cooled to -78 °C. Ozone was bubbled through the solution for 10 minutes. Dimethylsulfide (2.0 mL) was added and the reaction stirred until it reached rt at which time H<sub>2</sub>O was added the layers were separated. The aqueous layer was back extracted with CH<sub>2</sub>Cl<sub>2</sub> (2x). The combined organic layers were dried (MgSO<sub>4</sub>) filtered, and concentrated to give the crude product (0.091 g, 99%) as a yellow oil which was carried to the next step without further purification or characterization. The crude oil (0.091 g, 0.26 mmol, 1.0 equiv) in a 2 dram vial was dissolved in MeOH (3 mL). NaIO<sub>4</sub> (0.25 g, 1.2 mmol, 4.0 equiv) was dissolved in H<sub>2</sub>O (1 mL) and added to the reaction via a steady stream. That was allowed to stir for 24 h. The solution was filtered and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x). The combined organic layers were dried (MgSO<sub>4</sub>) filtered, and concentrated to give the crude product (0.015 g, 27%) as a white solid, which was carried on to the next step without further purification or characterization. The white solid was placed in a 2 dram vial and dissolved in a HCl/MeOH solution (1.0 mL, 1.25 M) and stirred at 60 °C for 3 h. Once the solution reached rt it was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x). The combined organic layers were dried (MgSO<sub>4</sub>) filtered, and concentrated to give the crude product. The crude product was purified via SiO<sub>2</sub> flash column chromatography (25 % EtOAc/hexanes) to yield 0.0145 g (84%) of the title

compound as a clear oil. The spectral data was consistent with that reported in the literature.<sup>99</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 2.72 (dd, *J* = 5.1, 17.3 Hz, 1H), 3.26 (dd, *J* = 10.3, 17.3 Hz, 1H), 3.70 (s, 6H), 4.09 (dd, *J* = 5.1, 10.3 Hz, 1H), 7.20-7.35 (5H, m); [α]<sub>D</sub><sup>23</sup> -77 (*c* 0.60, MeOH); Literature value [α]<sub>D</sub><sup>20</sup> -103 (*c* 1.19, MeOH).<sup>99</sup>

### 4.3 Chapter 2

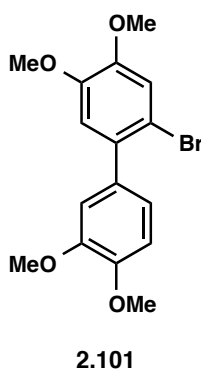


2.103

*2-Bromo-3',4,4',5-tetramethoxy-1,1'-biphenyl (2.103)*. 4-Bromoveratrole (20.0 g, 92.1 mmol, 1.00 equiv) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1000 mL) in a 2 L round-bottomed flask and cooled to -78 °C. To this solution was added dropwise (over 1 h) a pre-mixed solution of PIFA (21.8 g, 50.7 mmol, 0.55 equiv) and BF<sub>3</sub>•Et<sub>2</sub>O (12.7 mL, 101 mmol, 1.10 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (500 mL). The reaction mixture was stirred until the starting material was completely consumed (~1 h). The reaction was quenched by adding 10% NaOH (500 mL) and the reaction mixture was vigorously stirred for 1 h. The product was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and

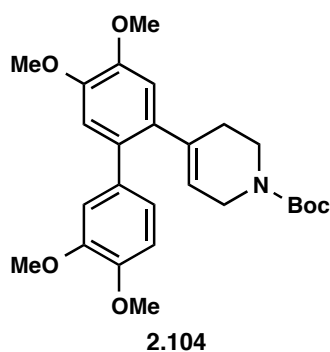


concentrated *in vacuo* and recrystallized from EtOH. The veratrole dimer was obtained as a white crystalline solid (18.8 g, 95%). Analytical data was in agreement with that reported.<sup>307</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.86 (s, 6H), 3.91 (s, 6H), 6.76 (s, 2H), 7.11 (s, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  56.26, 56.31, 114.04, 114.05, 115.20, 134.10, 148.12, 149.23.



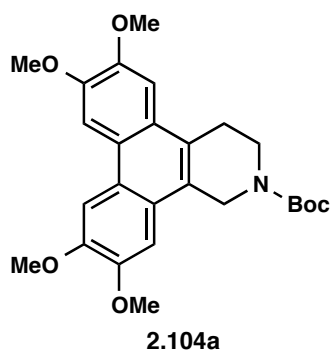
*2-Bromo-3',4,4',5-tetramethoxy-1,1'-biphenyl* (**2.101**) *2-Bromo-3',4,4',5-tetramethoxy-1,1'-biphenyl* (**2.103**, 10.0 g, 23.0 mmol, 1.00 equiv) was dissolved in anhydrous THF (400 mL) and cooled to  $-78$  °C. To this solution was added *n*-BuLi (9.30 mL, 23.0 mmol, 1.00 equiv, 2.5M in hexanes) dropwise via syringe pump (0.62 mL/min). Upon complete addition of *n*-BuLi the reaction was stirred for another 20 min before 2.0 mL of MeOH was added as a proton source. The quenched reaction mixture was stirred for 5 min at  $-78$

°C and then poured into a separatory funnel with 100 mL of H<sub>2</sub>O. The product was extracted with EtOAc (3x). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* and recrystallized from MeOH. The title compound was obtained as an off-white crystalline solid (6.79 g, 84%). Analytical data was in agreement with that reported. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.87 (s, 3H), 3.91 (s, 6H), 3.93 (s, 3H), 6.84 (s, 1H), 6.94 (m, 2H), 6.95 (s, 1H), 7.12 (s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 56.05, 56.13, 56.27, 56.41, 110.81, 112.84, 113.22, 114.10, 115.91, 121.86, 134.01, 134.72, 148.38, 148.40, 148.57, 148.79.



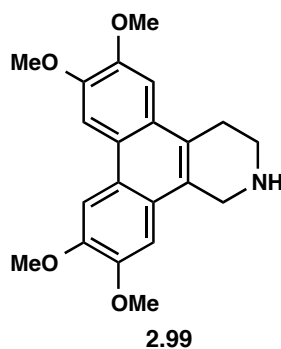
*tert*-Butyl 4-(3',4,4',5-Tetramethoxy-[1,1'-biphenyl]-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (**2.104**). 2-Bromo-3',4,4',5-tetramethoxy-1,1'-biphenyl (**2.101**, 6.74 g, 19.1 mmol, 1.10 equiv) was dissolved in anhydrous THF and cooled to -78 °C. To this solution was added *t*-BuLi (23.5 mL, 39.9 mmol, 2.30 equiv, 1.7 M in hexanes) dropwise via a syringe pump. Upon complete addition of *t*-BuLi the reaction was stirred for 30 min before *N*-boc-piperidone was added (3.46 g, 17.3 mmol, 1.0 equiv) dropwise in 30 mL THF. That solution was allowed to warm to rt while stirring slowly. Upon completion (~3

h) of the reaction, silica gel was added, stirred for 1 h, concentrated, loaded directly on to a column, and purified SiO<sub>2</sub> flash column chromatography (25% EtOAc/hexanes) to yield 6.64 g (84%) of the title compound as an orange oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.94 – 6.89 (m, 2H), 6.86 (d, *J* = 8.8 Hz, 1H), 6.82 (s, 1H), 6.75 (s, 1H), 5.87 – 5.52 (m, 1H), 4.03 – 3.94 (m, 2H), 3.91 (s, 3H) 3.89 (s, 3H), 3.89 (s, 3H) 3.84 (s, 3H), 3.29 (s, 2H), 2.00 – 1.79 (m, 2H), 1.44 (s, 9H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 154.96, 148.54, 148.10, 148.07, 147.88, 138.73, 134.47, 133.85, 131.96, 123.23, 121.09, 113.35, 112.83, 112.36, 111.02, 79.63, 56.14, 56.08, 56.06, 55.96, 55.94, 29.49, 28.54. IR (neat) 2933, 2836, 1694, 1603, 1505, 1464, 1417, 1248, 1211, 1173, 1151, 1028, 864,764 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>26</sub>H<sub>34</sub>NO<sub>6</sub><sup>+</sup>: 456.2386, found 456.2884.



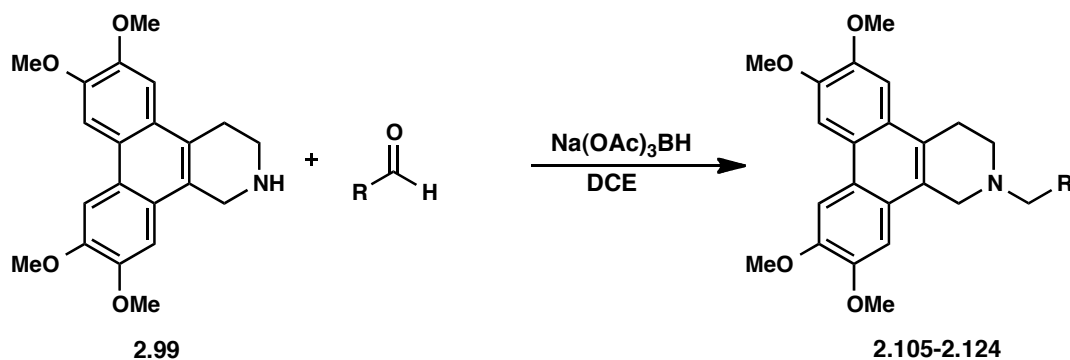
*tert-Butyl* 6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo-[f,h]-isoquinoline-2(1H)-carboxylate (**2.104a**). Aryl alkene **2.104** (6.53 g, 12.7 mmol, 1.0 equiv) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and cooled to -78 °C. Two drops of TFAA were then added to this solution. In a separate flask containing VOF<sub>3</sub> (3.45 g, 27.9 mmol, 2.2 equiv) was added anhydrous CH<sub>2</sub>Cl<sub>2</sub> (40 mL), anhydrous EtOAc (20 mL), TFA (1.5 mL) and TFAA

(2 drops). The VOF<sub>3</sub> solution was then added over 20 min to the aryl alkene solution. The reaction mixture was stirred until the starting material had been consumed (~1 h) and quenched with 10% NaOH (50 mL). The biphasic mixture was vigorously stirred for 1 h at rt. The aqueous layer was extracted (3x) with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layers were washed with brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Filtration and concentration *in vacuo* gave the crude product. Purification by SiO<sub>2</sub> flash column chromatography (50% EtOAc/hexanes) yielded 6.18 g (95%) of **5b** as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.81 (s, 2H), 7.28 (s, 1H), 7.17 (s, 1H), 4.96 (s, 2H), 4.12 (d, *J* = 3.5 Hz, 6H), 4.03 (d, *J* = 5.2 Hz, 6H), 3.87 (t, *J* = 5.7 Hz, 2H), 3.15 (t, *J* = 5.6 Hz, 2H), 1.54 (s, 9H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 155.06, 149.02, 148.93, 148.81, 148.76, 125.52, 123.96, 123.71, 123.55, 103.97, 103.53, 103.46, 102.88, 80.11, 56.18, 56.03, 55.98, 28.64, 28.55 IR (neat) 2972, 2834, 1690, 1620, 1514, 1471, 1424, 1248, 1169, 1150, 1047, 1021, 838, 767 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>26</sub>H<sub>32</sub>NO<sub>6</sub><sup>+</sup>: 454.2230, found 454.2209.



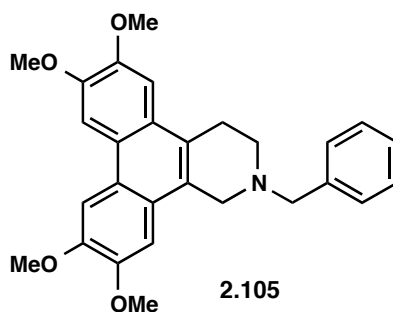
*6,7,10,11-Tetramethoxy-1,2,3,4-tetrahydrodibenzo-[f,h]-isoquinoline* (**2.99**). Piperidine **2.104b** (6.01 g, 13.3 mmol, 1.00 equiv) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (40 mL).

TFA (13 mL) was added to the solution. The reaction mixture immediately turned dark purple and slowly turned a light brown color. After the reaction was stirred for 1 h the solvent was removed by passing N<sub>2</sub> over it. To the remaining residue was added CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and NH<sub>3</sub> (10 mL, 2.0 M in MeOH). This solution was stirred for 30 min and then poured into a separatory funnel containing 10% NaOH (aq.). The product was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x) and combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Filtration and concentration *in vacuo* furnished 4.64 g (96%) the crude product as a yellow solid, which was used without further purification. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.78 (s, 2H), 7.23 (s, 1H), 7.05 (s, 1H), 4.32 (s, 2H), 4.11 (s, 3H), 4.10 (s, 3H), 4.02 (s, 3H), 3.99 (s, 3H), 3.31 (t, *J* = 5.9 Hz, 2H), 3.02 (t, *J* = 5.8 Hz, 2H), 2.17 (s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 148.81, 148.79, 148.60, 148.53, 126.96, 126.16, 125.98, 124.26, 123.54, 123.31, 103.71, 103.49, 103.41, 102.91, 56.15, 56.13, 55.95, 46.56, 43.67, 26.89; IR (neat) 3390, 2956, 2835, 1618, 1515, 1462, 1425, 1248, 1214, 1156, 1044, 1013, 843, 799, 764 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>21</sub>H<sub>24</sub>NO<sub>4</sub><sup>+</sup>: 354.1700, found 354.1698.



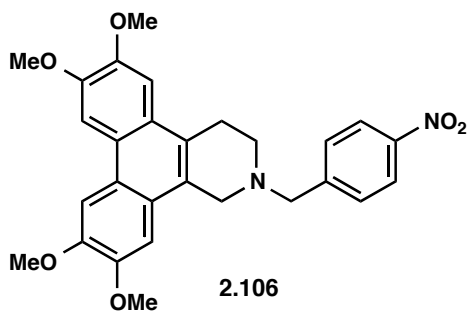
*Representative procedure for the reductive amination reaction:*

Phenanthropiperidine **2.99** (1.00 equiv, 0.05-0.08 mmol) and aldehyde (3.00 equiv) were dissolved in anhydrous DCE (0.02M) and allowed to stir for 5 min at rt. Na(OAc)<sub>3</sub>BH (5.00 equiv) was added in one portion (as a solid) and the reaction was allowed to stir at rt until completion (6-24 h, monitored via TLC). The reaction was quenched with 10% NaOH and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x). The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, concentrated, and purified via prep TLC (2.5-5% MeOH (2.0 M NH<sub>3</sub> in MeOH)/CH<sub>2</sub>Cl<sub>2</sub>).

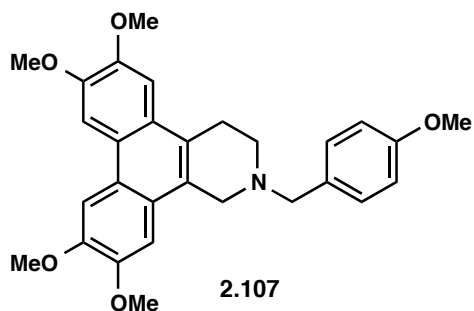


*2-Benzyl-6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline (2.105).* The title compound was isolated as an off-white solid (74%): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.81 (s, 2H), 7.53 (d, *J* = 3.9 Hz, 2H), 7.38 (d, *J* = 7.1 Hz, 3H), 7.25 (s, 1H), 6.99 (s, 1H), 4.34 (s, 2H), 4.16 (s, 2H), 4.11 (d, *J* = 3.3 Hz, 6H), 4.03 (s, 3H), 3.97 (s, 3H), 3.30 (s, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 149.08, 148.92, 133.04, 130.38, 130.23, 130.20, 128.92, 128.62, 128.40, 125.10, 124.66, 124.62, 123.99, 123.84, 103.95, 103.65, 103.48,

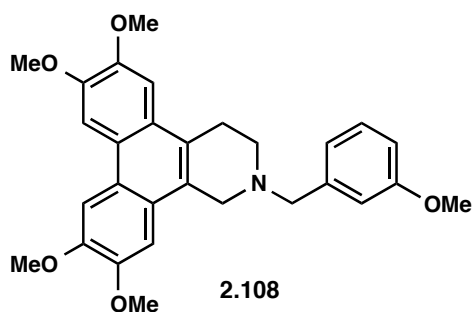
102.87, 59.98, 56.21, 56.19, 56.09, 56.03, 51.77, 48.13, 29.83; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{28}\text{H}_{30}\text{NO}_4^+$ : 444.2169, found 444.2165.



*6,7,10,11-Tetramethoxy-2-(4-nitrobenzyl)-1,2,3,4-tetrahydrobenzo-[f,h]-isoquinoline* (**2.106**). The title compound was isolated as an off-white solid (71%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.21 (d,  $J = 8.7$  Hz, 2H), 7.82 (s, 1H), 7.81 (s, 1H), 7.65 (d,  $J = 8.6$  Hz, 2H), 7.27 (s, 1H), 7.05 (s, 1H), 4.11 (s, 3H), 4.11 (s, 3H), 4.07 (s, 2H), 4.03 (s, 3H), 3.99 (s, 3H), 3.96 (s, 2H), 3.19 (t,  $J = 5.6$  Hz, 2H), 2.91 (t,  $J = 5.8$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  148.74, 148.71, 148.58, 148.47, 147.21, 146.64, 129.31, 125.75, 125.48, 125.21, 124.04, 123.61, 123.50, 123.42, 103.81, 103.46, 103.26, 102.82, 61.82, 55.99, 55.98, 55.90, 55.80, 54.42, 49.79, 27.14; IR (neat) 2917, 1516, 1344, 1248, 1146, 1046, 842  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{28}\text{H}_{29}\text{N}_2\text{O}_6^+$ : 489.2026, found 489.2030.

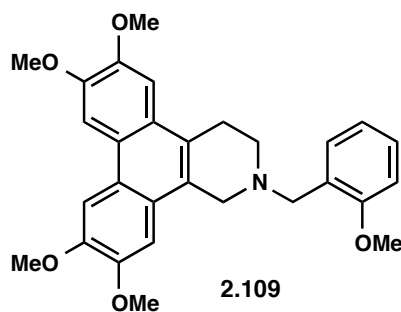


*6,7,10,11-Tetramethoxy-2-(4-methoxybenzyl)-1,2,3,4-tetrahydrodibenzo[f,h]-isoquinoline* (**2.107**). The title compound was isolated as an off-white solid (81%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.82 (d,  $J = 2.6$  Hz, 2H), 7.39 (d,  $J = 8.6$  Hz, 2H), 7.27 (s, 1H), 7.10 (s, 1H), 6.91 (d,  $J = 8.6$  Hz, 2H), 4.12 (dd,  $J = 6.3, 1.2$  Hz, 6H), 4.03 (dd,  $J = 12.0, 5.1$  Hz, 8H), 3.84 – 3.80 (m, 5H), 3.17 (t,  $J = 5.5$  Hz, 2H), 2.90 (t,  $J = 5.7$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  158.96, 148.79, 148.76, 148.58, 148.48, 130.60, 130.45, 128.76, 126.17, 125.83, 124.44, 123.59, 123.51, 114.05, 113.85, 104.01, 103.53, 103.37, 103.11, 65.18, 62.28, 56.15, 56.03, 55.96, 55.44, 54.50, 49.54, 27.33; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{29}\text{H}_{32}\text{NO}_5^+$ : 474.2280, found 474.2285.



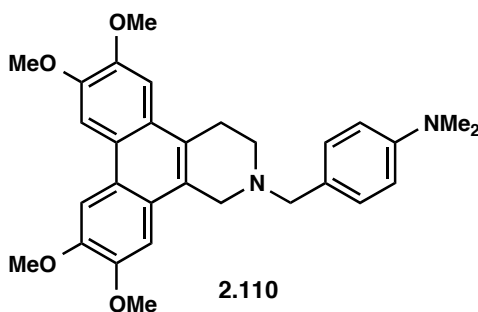


*6,7,10,11-Tetramethoxy-2-(3-methoxybenzyl)-1,2,3,4-tetrahydrodibenzo[*f,h*]isoquinoline* (**2.108**). The title compound was isolated as an off-white solid (85%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.80 (d,  $J = 1.6$  Hz, 2H), 7.25 (d,  $J = 6.1$  Hz, 2H), 7.08 (s, 1H), 7.04 (d,  $J = 6.7$  Hz, 2H), 6.88 – 6.79 (m, 1H), 4.09 (d,  $J = 1.0$  Hz, 6H), 4.04 (s, 2H), 4.00 (d,  $J = 5.7$  Hz, 6H), 3.84 (s, 2H), 3.80 (s, 3H), 3.15 (d,  $J = 5.0$  Hz, 2H), 2.89 (t,  $J = 5.6$  Hz, 2H).;  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  159.94, 148.84, 148.81, 148.63, 148.53, 140.32, 129.43, 126.15, 125.85, 125.82, 124.43, 123.63, 123.54, 121.55, 114.49, 112.95, 104.06, 103.60, 103.45, 103.14, 62.81, 56.17, 56.16, 56.04, 55.97, 55.38, 54.59, 49.68, 27.32; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841  $\text{cm}^{-1}$ ;  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{29}\text{H}_{32}\text{NO}_5^+$ : 474.2280, found 474.2269.

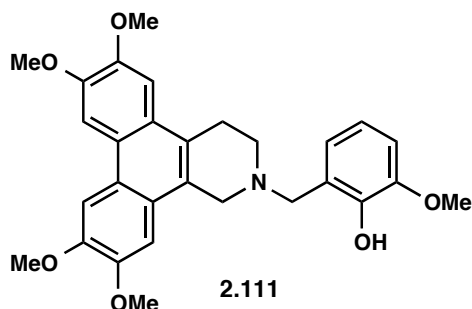


*6,7,10,11-Tetramethoxy-2-(2-methoxybenzyl)-1,2,3,4-tetrahydrodibenzo[*f,h*] isoquinoline* (**2.109**). The title compound was isolated as an off-white solid (56%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.82 (s, 1H), 7.81 (s, 1H), 7.53 (d,  $J = 7.8$  Hz, 1H), 7.32 – 7.27 (m, 1H), 7.28 (s, 1H), 7.10 (s, 1H), 7.01 – 6.87 (m, 2H), 4.16 (bs, 2H), 4.11 (s, 3H), 4.11 (s, 3H), 4.03 (s, 3H), 4.01 (s, 3H), 4.01 (bs, 2H), 3.87 (s, 3H), 3.22 (t,  $J = 5.1$  Hz, 2H), 3.06 (bs, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  157.86, 148.65, 148.61, 148.42, 148.31, 130.45,

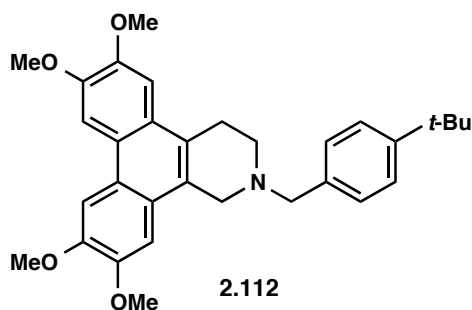
128.18, 126.33, 125.96, 125.73, 124.35, 123.43, 123.34, 120.47, 110.38, 103.92, 103.43, 103.30, 102.99, 56.01, 56.01, 55.88, 55.83, 55.81, 55.42, 54.14, 49.83, 27.16; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{29}\text{H}_{32}\text{NO}_5^+$ : 474.2280, found 474.2269.



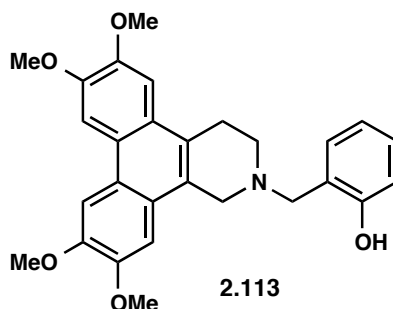
*N,N*-Dimethyl-4-((6,7,10,11-tetramethoxy-3,4-dihydrodibenzo[*f,h*]isoquinolin-2(1*H*)-yl)methyl)aniline (**2.110**). The title compound was isolated as an off-white solid (73%):  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  7.81 (d,  $J = 1.3$  Hz, 2H), 7.29 (d,  $J = 8.6$  Hz, 2H), 7.26 (s, 1H), 7.08 (s, 1H), 6.73 (d,  $J = 8.7$  Hz, 2H), 4.04 (d,  $J = 1.2$  Hz, 6H), 4.01 (s, 2H), 3.95 (d,  $J = 5.3$  Hz, 6H), 3.77 (s, 2H), 3.13 (t,  $J = 5.6$  Hz, 2H), 2.92 (s, 6H), 2.90 – 2.85 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  150.77, 149.49, 149.45, 149.30, 149.20, 130.73, 128.97, 126.47, 126.09, 124.72, 123.93, 123.85, 112.95, 112.64, 104.68, 104.18, 104.04, 103.78, 65.56, 62.44, 56.47, 56.45, 56.29, 56.23, 49.76, 41.03, 40.94, 30.25, 27.49; IR (neat) 2919, 1615, 1515, 1470, 1248, 1146, 1045, 840  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{30}\text{H}_{35}\text{N}_2\text{O}_4^+$ : 487.2597, found 487.2604.



*2-Methoxy-6-((6,7,10,11-tetramethoxy-3,4-dihydrodibenzo[*f,h*]isoquinolin-2(1*H*)-yl)methyl)phenol (2.111)*. The title compound was isolated as an off-white solid (67%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.82 (s, 1H), 7.81 (s, 1H), 7.24 (s, 1H), 7.05 (s, 1H), 6.92 – 6.84 (m, 1H), 6.81 (t,  $J = 7.7$  Hz, 1H), 6.75 (d,  $J = 7.1$  Hz, 1H), 4.20 (s, 2H), 4.11 (d,  $J = 1.4$  Hz, 8H), 4.02 (s, 3H), 4.01 (s, 3H), 3.87 (s, 3H), 3.25 (d,  $J = 4.7$  Hz, 2H), 3.06 (s, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  148.97, 148.91, 148.76, 148.18, 147.41, 125.57, 125.37, 124.12, 123.99, 123.77, 123.67, 121.22, 121.12, 119.03, 111.35, 103.96, 103.67, 103.45, 102.98, 60.48, 56.19, 56.14, 56.03, 55.96, 53.66, 48.85, 26.48; IR (neat) 2933, 1619, 1514, 1474, 1249, 1144, 1044, 842  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{29}\text{H}_{32}\text{NO}_6^+$ : 490.2230, found 490.2230.

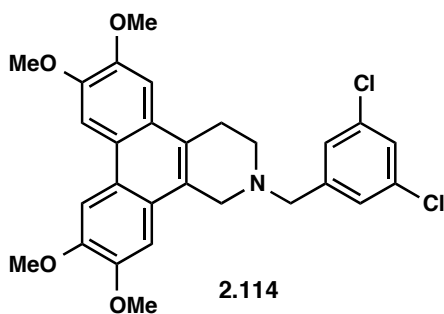


2-(4-(*tert*-Butyl)benzyl)-6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[*f,h*]-isoquinoline (**2.112**). The title compound was isolated as an off-white solid (81%): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.81 (s, 1H), 7.81 (s, 1H), 7.40 (s, 4H), 7.26 (s, 1H), 7.08 (s, 1H), 4.11 (s, 3H), 4.11 (s, 3H), 4.08 (s, 2H), 4.02 (s, 3H), 4.00 (s, 3H), 3.88 (s, 2H), 3.18 (t, *J* = 5.4 Hz, 2H), 2.95 (t, *J* = 5.3 Hz, 2H), 1.34 (s, 9H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 150.45, 148.82, 148.80, 148.64, 148.53, 138.12, 129.14, 126.99, 125.72, 125.59, 125.43, 124.35, 123.65, 123.55, 104.01, 103.58, 103.43, 103.11, 65.27, 56.15, 56.01, 55.93, 54.29, 49.37, 34.65, 31.53, 26.96; IR (neat) 2959, 1515, 1479, 1248, 1147, 1046, 839 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>32</sub>H<sub>38</sub>NO<sub>4</sub><sup>+</sup>: 500.2801, found 500.2789.

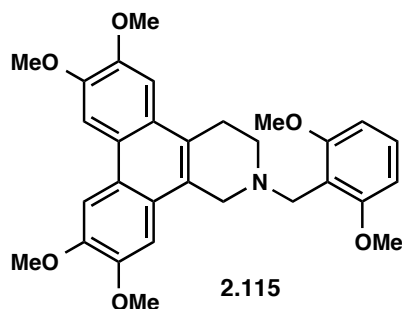


2-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f,h*]isoquinolin-2(1H)-yl)methyl) phenol (**2.113**). The title compound was isolated as an off-white solid (65%): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.83 (s, 1H), 7.82 (s, 1H), 7.25 (s, 1H), 7.25 – 7.19 (m, 1H), 7.10 (d, *J* = 7.3 Hz, 1H), 7.05 (s, 1H), 6.89 – 6.82 (m, *J* = 12.2, 4.7 Hz, 2H), 4.16 (bs, 2H), 4.12 (s, 3H), 4.12 (s, 3H), 4.09 (bs, 2H), 4.02 (s, 3H), 4.02 (s, 3H), 3.23 (t, *J* = 5.2 Hz, 2H), 3.03 (bs, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 158.15, 148.97, 148.90, 148.77, 129.10, 128.94, 125.61, 125.38, 124.37, 123.99, 123.77, 123.66, 121.28, 119.33, 116.48, 103.97, 103.66,

103.45, 102.96, 61.16, 56.20, 56.19, 56.13, 55.97, 53.85, 48.95, 26.73; IR (neat) 1619, 1514, 1249, 1143, 1045, 845  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{28}\text{H}_{30}\text{NO}_5^+$ : 460.2124, found 460.2137.

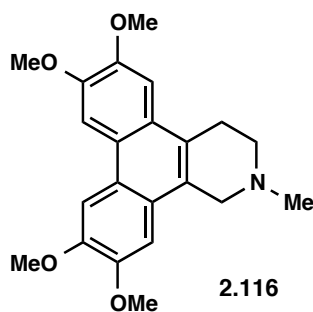


*2-(3,5-Dichlorobenzyl)-6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[f,h]-isoquinoline (2.114)*. The title compound was isolated as an off-white solid (81%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.80 (s, 2H), 7.36 (s, 2H), 7.28 (s, 1H), 7.17 (d,  $J = 18.9$  Hz, 1H), 7.04 (s, 1H), 4.10 (s, 6H), 4.01 (d,  $J = 6.7$  Hz, 8H), 3.80 (s, 2H), 3.17 (s, 2H), 2.89 (d,  $J = 5.2$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  148.88, 148.86, 148.71, 148.59, 142.52, 135.09, 127.52, 127.34, 125.95, 125.68, 125.40, 124.93, 124.24, 123.66, 123.56, 104.00, 103.62, 103.43, 102.99, 61.79, 56.16, 56.05, 55.97, 54.47, 49.89, 27.30; IR (neat) 2932, 1619, 1569, 1514, 1425, 1249, 1147, 846  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{28}\text{H}_{28}\text{NO}_4\text{Cl}_2^+$ : 512.1395, found 512.1400.



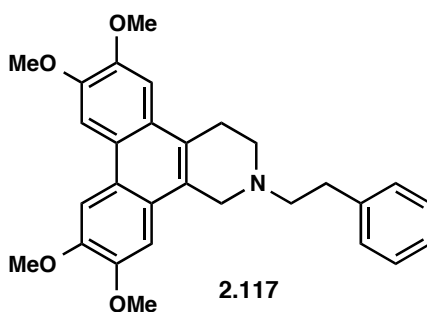
*2-(2,6-Dimethoxybenzyl)-6,7,10,11-tetramethoxy-1,2,3,4-tetrahydrodibenzo[f,h]*

*isoquinoline* (**2.115**). The title compound was isolated as an off-white solid (55%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.81 (s, 1H), 7.80 (s, 1H), 7.27 (s, 1H), 7.24 (d,  $J = 8.3$  Hz, 1H), 7.15 (s, 1H), 6.61 (d,  $J = 8.4$  Hz, 2H), 4.10 (t,  $J = 2.3$  Hz, 8H), 4.04 (s, 5H), 4.02 (s, 3H), 3.86 (s, 6H), 3.16 (d,  $J = 5.4$  Hz, 2H), 3.08 (t,  $J = 5.6$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  159.65, 148.72, 148.60, 148.42, 148.29, 128.98, 126.50, 125.96, 125.92, 124.65, 123.47, 123.42, 114.20, 104.07, 103.89, 103.55, 103.44, 103.15, 56.14, 55.93, 55.91, 55.82, 53.25, 49.94, 48.91, 27.05; IR (neat) 2928, 1514, 1467, 1247, 1147, 1047, 841  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{30}\text{H}_{34}\text{NO}_6^+$ : 504.2386, found 504.2380.



*6,7,10,11-Tetramethoxy-2-methyl-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline* (2.116).

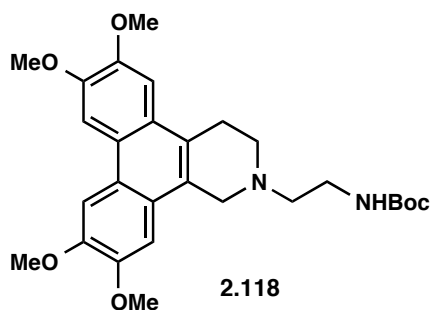
The title compound was isolated as an off-white solid (71%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.83 (s, 1H), 7.83 (s, 1H), 7.30 (s, 1H), 7.14 (s, 1H), 4.12 (s, 6H), 4.05 (s, 3H), 4.05 (s, 3H), 3.95 (s, 2H), 3.22 (t,  $J = 5.8$  Hz, 2H), 2.90 (t,  $J = 5.9$  Hz, 2H), 2.66 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  148.88, 148.66, 148.55, 125.79, 125.75, 125.64, 124.32, 123.63, 123.54, 104.08, 103.63, 103.49, 103.10, 56.19, 56.13, 56.07, 56.01, 52.51, 46.58, 27.51; IR (neat) 3373, 2975, 1515, 1425, 1366, 1249, 1150, 844  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{22}\text{H}_{26}\text{NO}_4^+$ : 368.1862, found 368.1869.



*6,7,10,11-Tetramethoxy-2-phenethyl-1,2,3,4-tetrahydrodibenzo[f,h]isoquinoline* (2.117).

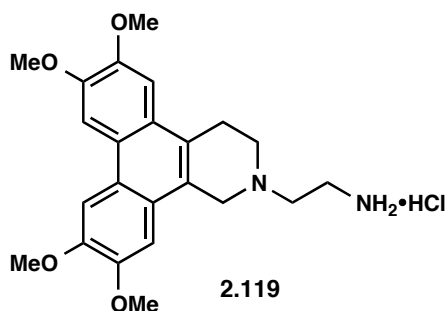
The title compound was isolated as an off-white solid (76%):  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  7.83 (s, 1H), 7.82 (s, 1H), 7.32 (dd,  $J = 5.0, 1.5$  Hz, 4H), 7.29 (s, 1H), 7.24 – 7.19 (m, 1H), 7.09 (s, 1H), 4.06 (d,  $J = 1.5$  Hz, 8H), 3.99 (d,  $J = 8.9$  Hz, 6H), 3.21 (t,  $J = 5.6$  Hz, 2H), 3.09 – 2.98 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  149.52, 149.50, 149.34, 149.21, 141.23, 129.32, 128.93, 126.58, 126.40, 126.03, 124.67, 123.94, 123.85, 104.67, 104.20, 104.04, 103.66, 60.45, 56.47, 56.46, 56.36, 56.26, 50.59, 34.25, 27.54;

IR (neat) 1615, 1513, 1422, 1248, 1149, 1042, 840  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{29}\text{H}_{32}\text{NO}_4^+$ : 458.2331, found 458.2329.



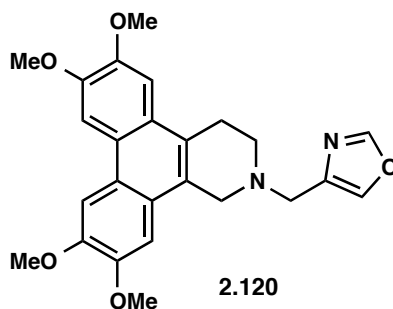
*tert-Butyl* (2-(6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f,h*]isoquinolin-2(1*H*)-yl)ethyl)carbamate (**2.118**). The title compound was isolated as an off-white solid (95%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.80 (s, 1H), 7.80 (s, 1H), 7.27 (s, 1H), 7.10 (s, 1H), 4.10 (s, 6H), 4.03 (s, 6H), 4.00 (s, 2H), 3.45 (d,  $J = 5.0$  Hz, 2H), 3.18 (d,  $J = 5.1$  Hz, 2H), 2.95 (t,  $J = 5.6$  Hz, 2H), 2.84 (t,  $J = 5.7$  Hz, 2H), 1.48 – 1.43 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  156.24, 148.88, 148.71, 148.59, 125.94, 125.59, 125.35, 124.23, 123.64, 123.52, 103.97, 103.59, 103.43, 102.93, 79.30, 57.15, 56.15, 56.07, 55.98, 54.08, 49.98, 37.64, 29.81, 28.55, 28.49, 28.45, 28.10, 27.23; IR (neat) 3373, 2975, 1701, 1515, 1425, 1366, 1249, 1150, 844  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{28}\text{H}_{37}\text{N}_2\text{O}_6^+$ : 497.2652, found 497.2665.





*2-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[f,h]isoquinolin-2(1H)-yl)ethanamine*

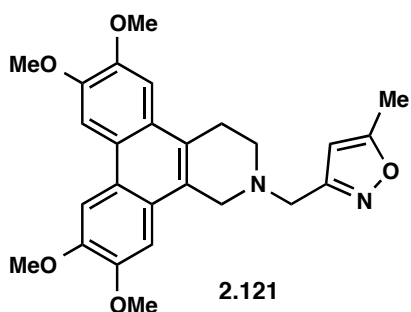
**(2.119)**. The title compound was isolated as an off-white solid (84%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.81 (s, 1H), 7.80 (s, 1H), 7.26 (s, 1H), 7.07 (s, 1H), 4.10 (t,  $J = 5.6$  Hz, 10H), 4.05 (s, 6H), 3.69 (d,  $J = 5.1$  Hz, 2H), 3.22 (s, 2H), 3.03 (d,  $J = 21.2$  Hz, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  149.08, 149.07, 148.99, 148.83, 125.29, 123.92, 123.80, 123.67, 103.92, 103.67, 103.46, 102.77, 56.22, 56.19, 56.13, 56.03, 55.39, 53.84, 49.72, 36.61, 29.84.; IR (neat) 3373, 2975, 1701, 1515, 1425, 1366, 1249, 1150, 844  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{23}\text{H}_{29}\text{N}_2\text{O}_4^+$ : 397.2122, found 397.2135.



*4-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[f,h]isoquinolin-2(1H)-yl)methyl)oxazole*

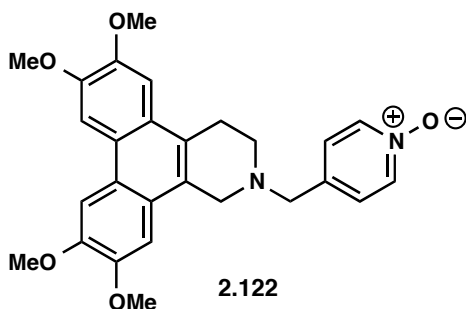
**(2.120)**. The title compound was isolated as an off-white solid (91%):  $^1\text{H}$  NMR (400

MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 7.91 (s, 1H), 7.82 (d, *J* = 2.1 Hz, 2H), 7.73 (s, 1H), 7.28 (s, 1H), 7.12 (s, 1H), 4.06 (d, *J* = 1.5 Hz, 8H), 3.99 (s, 3H), 3.97 (s, 3H), 3.85 (s, 2H), 3.17 (t, *J* = 5.9 Hz, 2H), 2.99 (t, *J* = 5.8 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 151.67, 149.49, 149.47, 149.30, 149.20, 138.17, 137.20, 126.42, 126.14, 126.09, 124.70, 123.91, 123.83, 104.67, 104.19, 104.05, 103.75, 56.47, 56.45, 56.33, 56.24, 50.48, 27.77; IR (neat) 2935, 1620, 1514, 1425, 1248, 1146, 1045, 843 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>25</sub>H<sub>27</sub>N<sub>2</sub>O<sub>5</sub><sup>+</sup>: 435.1920, found 435.1911.

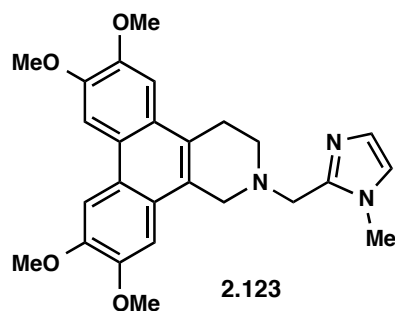


*5-Methyl-3-((6,7,10,11-tetramethoxy-3,4-dihydrodibenzo[f,h]isoquinolin-2(1H)-yl)methyl)isoxazole (2.121)*. The title compound was isolated as an off-white solid (72%): <sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 7.82 (s, 1H), 7.82 (s, 1H), 7.27 (s, 1H), 7.10 (s, 1H), 6.12 (d, *J* = 1.1 Hz, 1H), 4.08 – 4.03 (m, 8H), 3.98 (s, 3H), 3.97 (s, 3H), 3.89 (s, 2H), 3.25 – 3.10 (m, 2H), 2.94 (t, *J* = 5.8 Hz, 2H), 2.41 (d, *J* = 0.9 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 170.20, 162.48, 149.51, 149.34, 149.24, 126.36, 126.04, 125.99, 124.63, 123.94, 123.84, 104.64, 104.19, 104.04, 103.72, 102.22, 56.46, 56.45, 56.37, 56.24,

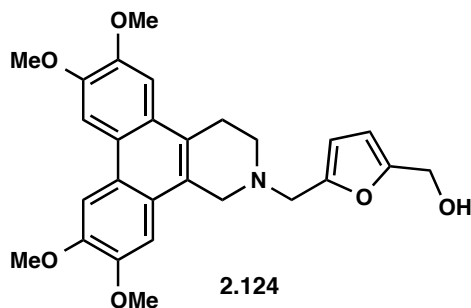
54.60, 50.44, 27.77, 12.62; IR (neat) 2918, 1607, 1514, 1424, 1248, 1145, 1046, 841  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{26}\text{H}_{29}\text{N}_2\text{O}_5^+$ : 449.2076, found 449.2062.



*4-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[f,h]isoquinolin-2(1H)-yl)methyl)pyridine 1-Oxide (2.122)*. The title compound was isolated as an off-white solid (55%):  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  8.13 (d,  $J = 6.3$  Hz, 2H), 7.83 (s, 2H), 7.40 (d,  $J = 6.4$  Hz, 2H), 7.29 (s, 1H), 7.06 (s, 1H), 4.13 – 3.92 (m, 14H), 3.83 (s, 2H), 3.19 (d,  $J = 5.9$  Hz, 2H), 2.91 (t,  $J = 5.9$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  149.57, 149.53, 149.40, 149.29, 139.41, 138.80, 126.51, 126.40, 126.00, 125.85, 124.56, 123.97, 123.88, 104.61, 104.22, 104.05, 103.63, 60.95, 56.47, 56.45, 56.35, 56.25, 54.75, 50.48, 27.77; IR (neat) 2918, 1620, 1514, 1474, 1249, 1146, 1043, 843  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{27}\text{H}_{29}\text{N}_2\text{O}_5^+$ : 461.2076, found 461.2077.



*6,7,10,11-Tetramethoxy-2-((1-methyl-1H-imidazol-2-yl)methyl)-1,2,3,4-tetrahydrodibenzo[*f,h*]isoquinoline (2.123)*. The title compound was isolated as an off-white solid (62%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.83 (s, 1H), 7.82 (s, 1H), 7.28 (s, 1H), 7.11 (s, 1H), 6.99 (d,  $J = 1.3$  Hz, 1H), 6.89 (d,  $J = 1.3$  Hz, 1H), 4.12 (s, 3H), 4.11 (s, 3H), 4.03 (s, 7H), 3.98 (s, 3H), 3.75 (s, 3H), 3.17 (t,  $J = 5.8$  Hz, 2H), 2.94 (t,  $J = 5.8$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  148.88, 148.83, 148.66, 148.56, 145.05, 127.24, 126.04, 125.68, 125.65, 124.30, 123.61, 123.44, 121.97, 103.97, 103.51, 103.38, 103.04, 56.17, 56.09, 55.97, 55.14, 54.35, 49.83, 33.19, 27.44; IR (neat) 2917, 1620, 1514, 1425, 1248, 1147, 1044  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{26}\text{H}_{30}\text{N}_3\text{O}_4^+$ : 448.2236, found 448.2223.



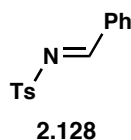
(5-((6,7,10,11-Tetramethoxy-3,4-dihydrodibenzo[*f,h*]isoquinolin-2(1*H*)-yl)methyl) furan-2-yl)methanol (**2.124**). The title compound was isolated as an off-white solid (72%): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.80 (s, 1H), 7.79 (s, 1H), 7.24 (s, 1H), 7.07 (s, 1H), 6.30 (d, *J* = 3.1 Hz, 1H), 6.26 (d, *J* = 3.1 Hz, 1H), 4.60 (s, 2H), 4.10 (s, 3H), 4.10 (s, 3H), 4.04 (bs, 2H), 4.02 (s, 3H), 4.01 (s, 3H), 3.88 (s, 2H), 3.18 (t, *J* = 5.4 Hz, 2H), 2.98 (t, *J* = 5.8 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 154.04, 151.75, 148.70, 148.67, 148.51, 148.40, 125.65, 125.51, 125.13, 124.14, 123.48, 123.40, 109.79, 108.38, 103.85, 103.46, 103.29, 102.90, 57.53, 56.02, 55.91, 55.83, 54.64, 53.64, 49.59, 26.82; IR (neat) 3393, 1619, 1514, 1425, 1248, 1146, 1012 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>27</sub>H<sub>30</sub>NO<sub>6</sub><sup>+</sup>: 464.2073, found 464.2055.

### Anti-proliferation assay

To improve solubility, each phenanthroindolizidine alkaloid was converted to its HCl salt by dissolving the free amine in CH<sub>2</sub>Cl<sub>2</sub>, adding a large excess (>10 equiv) of HCl (2.0 M in diethyl ether) and removing the solvent *in vacuo*. Stock solutions (10 mM) of the each compound were prepared in DMSO.

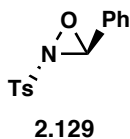
MCF-7, DU-145 and A549/NF-κB-luc cancer cells were harvested (125 G centrifuge for 5 min) from an exponential-phase maintenance culture. The cells were re-suspended in new culture media (RPMI1640 (GIBCO11875) with 10% FBS, EMEM (ATCC P/N 30-2003) with 10% FBS, and DMEM (ATCC P/N 30-2002) with 10% FBS, penicillin, streptomycin and hygromycin) and the cell density was adjusted to 10<sup>5</sup> cells/mL and dispensed in 96-well culture plates at a density of 5,000 cells per well (50 μL). The cells were incubated overnight to allow cells to adhere to the wells. Fresh

culture medium (50  $\mu$ L) containing varying concentrations of the test and control compounds was added to each well. The cultures were grown for an additional 72 h and alamarBlue® (10  $\mu$ L) was added. After 1.5 h, the fluorescence excitation (530 nm) and emission (590 nm) of each well were measured to determine the optical density. Each compound was tested in triplicate with less than 5% variation. Compound **2.112** was tested in duplicate.

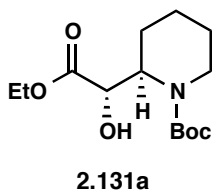


*(E)*-*N*-Benzylidene-4-methylbenzenesulfonamide (**2.128**). *p*-Toluenesulfonamide (50.45 g, 294.7 mmol, 1.00 equiv), Amberlyst-15 ion exchange resin (2.52 g, 0.05 equiv based on amount of sulfonamide), benzaldehyde (29.95 mL, 294.7 mmol, 1.00 equiv), and 4 Å molecular sieves (50.45 g, quantity same weight as sulfonamide) were stirred in dry toluene (600 mL). The reaction mixture was heated at reflux for 24 hours. The reaction mixture was allowed to cool to rt while stirring. The mixture was filtered to remove any insoluble materials and was then concentrated under reduced pressure. The resultant residue solidified upon standing and was recrystallized with EtOAc/hexanes (1/3) to yield 68.0 (89%) grams of the title compound as an off solid. The spectral data was identical to that reported in the literature.<sup>309</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.03 (s, 1H), 8.00 – 7.85 (m, 4H), 7.66 – 7.56 (m, 1H), 7.48 (dd,  $J$  = 8.4, 7.1 Hz, 2H), 7.34 (d,  $J$  = 8.1 Hz, 2H),

2.43 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.21, 144.69, 135.19, 135.01, 132.42, 131.35, 129.88, 129.21, 128.15, 21.72.

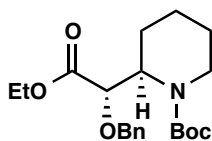


*(S)*-3-Phenyl-2-tosyl-1,2-oxaziridine (**2.129**). Imine **2.128** (68.0 g, 262 mmol, 1.00 equiv) and BTEAC (5.97 g, 26.2 mmol, 0.10 equiv) were dissolved in 350 mL  $\text{CHCl}_3$ . Sat. aq.  $\text{NaHCO}_3$  (400 mL) was added to the stirring solution. A solution of *m*-CPBA (70.5 g, 315 mmol, 1.2 equiv) in 300 mL  $\text{CHCl}_3$  was added dropwise to the vigorously stirring reaction mixture. After completion (3 h), the organic phase is separated and washed with water, 10% sodium sulfite, water, and brine. The organic layer was then dried ( $\text{MgSO}_4$ ), concentrated under reduced pressure (water bath can not rise above 40 °C), and recrystallized (Pentane/EtOAc) to give a white solid. The spectral data is identical to that reported in the literature.<sup>309</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.00 – 7.90 (m, 2H), 7.52 – 7.37 (m, 7H), 5.47 (s, 1H), 2.50 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  146.43, 131.48, 131.39, 130.56, 130.06, 129.42, 128.73, 128.23, 21.84.



*(R)*-*tert*-Butyl 2-((*S*)-2-Ethoxy-1-hydroxy-2-oxoethyl)piperidine-1-carboxylate (**2.131a**).

THF (1 mL) was added to a flame dried vial and cooled to  $-78\text{ }^{\circ}\text{C}$  under a  $\text{N}_2$  atmosphere. NaHMDS (0.37 mL, 0.37 mmol, 1.0M in THF, 1.50 equiv) was then added. Ester **2.130** (0.0708 g, 0.246 mmol, 1.00 equiv, in 1 mL THF) was added dropwise over 5 minutes. The reaction stirred at  $-78\text{ }^{\circ}\text{C}$  for 30 minutes and oxaziridine **2.129** (0.102 g, 0.370 mmol, 1.50 equiv, in 1 mL THF) was added via cannulation and stirred at  $-78$  for 1 hour. The reaction mixture was quenched with  $\text{NH}_4\text{Cl}$  and allowed to warm to rt. The organic layer was removed under reduced pressure and the mixture was diluted with  $\text{Et}_2\text{O}$  and the aqueous layer was separated, and washed with  $\text{Et}_2\text{O}$  (3X). The combined organic layers were dried ( $\text{MgSO}_4$ ), filtered, concentrated under reduced pressure. The crude reaction mixture was purified via  $\text{SiO}_2$  flash column chromatography (40%  $\text{EtOAc}$ /hexanes) to give 0.057 g (80%) the  $\alpha$ -hydroxy ester as a yellow oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.05 (bs, 1H), 4.30 (t,  $J = 7.3$  Hz, 1H), 4.26 – 4.01 (m, 2H), 3.94 (s, 1H), 2.94 – 2.83 (m, 2H), 2.00 – 1.85 (m, 1H), 1.60 – 1.42 (m, 5H), 1.36 (s, 9H), 1.22 (d,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.84, 154.76, 79.84, 60.76, 58.21, 51.76, 50.71, 39.83, 28.41, 27.71, 25.12, 21.58, 21.48, 19.02, 13.81.

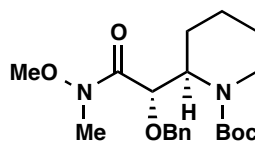


**2.132**



*(R)*-*tert*-Butyl 2-((*S*)-1-(Benzyloxy)-2-ethoxy-2-oxoethyl)piperidine-1-carboxylate (**2.132**).

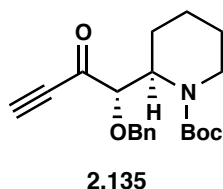
To a suspension of sodium hydride (0.024 g, 0.60 mmol, 60% in dispersion oil, 3.00 equiv) in THF at 0 °C, a solution of  $\alpha$ -hydroxy ester **2.131a** (0.057 g, 0.2 mmol, 1.00 equiv) in THF (1 mL) dropwise. The mixture was stirred at 0 °C for 15 minutes and cooled to -15 °C. Benzyl bromide (0.051 g, 0.035 mL, 0.30 mmol) was added in a steady stream via syringe. Upon completion (monitored via TLC, 2 h) NaHCO<sub>3</sub> (sat. *aq.*) was added carefully and allowed to warm to rt. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and separated and washed with H<sub>2</sub>O (2X). The aqueous layers were then back extracted with CH<sub>2</sub>Cl<sub>2</sub> (3X). The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated. The crude material was then purified via SiO<sub>2</sub> flash column chromatography (25% EtOAc/hexanes) to give 0.061 g (80%) of the title compound as a yellow oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.38 – 7.28 (m, 5H), 4.71 (d, *J* = 11.7 Hz, 1H), 4.46 – 4.27 (m, 2H), 4.24 – 4.13 (m, 3H), 2.85 (s, 1H), 2.04 – 1.93 (m, 1H), 1.62 – 1.46 (m, 4H), 1.42 (s, 11H), 1.27 (t, *J* = 7.1 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.34, 154.55, 135.67, 128.42, 128.37, 128.21, 72.58, 60.89, 50.47, 28.34, 24.88, 19.14, 14.18.



**2.133**

*(R)*-*tert*-Butyl 2-((*S*)-1-(Benzyloxy)-2-(methoxy(methyl)amino)-2-oxoethyl)piperidine-1-carboxylate (**2.133**).  $\alpha$ -Hydroxy ester (**2.132**, 0.061 g, 0.16 mmol, 1.00 equiv) and *N,O*-Dimethylhydroxylamine (0.024 g, 0.25 mmol, 1.55 equiv) were slurried in THF and

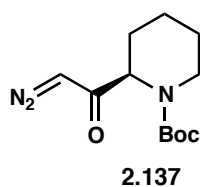
cooled to  $-20\text{ }^{\circ}\text{C}$ . A solution of *i*-propylmagnesium chloride (0.24 mL, 0.48 mmol, 3.00 equiv) was added dropwise maintaining a temperature below  $-10\text{ }^{\circ}\text{C}$ . Upon completion, as judged via TLC (5 h), the reaction was quenched with sat. aq.  $\text{NH}_4\text{Cl}$ , extracted with  $\text{Et}_2\text{O}$  (3x), dried ( $\text{MgSO}_4$ ), concentrated under reduced pressure, and purified via  $\text{SiO}_2$  flash column chromatography (40%  $\text{EtOAc}$ /hexanes) to afford 0.017 g (27%) of the Weinreb amide as a colorless oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.31 – 7.20 (m, 5H), 4.73 – 4.24 (m, 4H), 3.95 (t,  $J = 30.7$  Hz, 1H), 3.50 (s, 3H), 3.13 (s, 3H), 2.83 (s, 1H), 1.35 (m, 15H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.13, 154.76, 137.50, 128.46, 128.35, 128.06, 127.95, 79.52, 72.09, 61.29, 40.38, 32.65, 28.45, 25.21, 25.16, 19.49; IR (neat) 2935, 1689, 1665, 1411, 1365, 1164, 700  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{21}\text{H}_{33}\text{N}_2\text{O}_5^+$ : 393.2384, found 393.2377;  $[\alpha]_{\text{D}}^{23} +22$  ( $c$  1.0,  $\text{CHCl}_3$ ).



*(R)*-*tert*-Butyl 2-((*S*)-1-(Benzyloxy)-2-oxobut-3-yn-1-yl)piperidine-1-carboxylate (**2.135**).

Weinreb amide **2.133** (0.017 g, 0.043 mmol, 1.00 equiv) was dissolved in 1 mL of dry THF and cooled to  $0\text{ }^{\circ}\text{C}$ . Ethynylmagnesium bromide (0.42 mL, 0.21 mmol, 0.5M in THF, 5.00 equiv) was added dropwise via an addition funnel at  $0\text{ }^{\circ}\text{C}$ . Upon completion, monitored by TLC (3 h), the reaction was quenched with cold 10% HCl. The mixture was extracted with ether (3x), dried with  $\text{MgSO}_4$ , and concentrated under reduced pressure. The crude material was purified via  $\text{SiO}_2$  flash column chromatography (40%

EtOAc/hexanes) to give 0.015 g (97%) of the title compound as an orange oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.34 (qd,  $J = 5.9, 2.3$  Hz, 5H), 4.78 (d,  $J = 11.5$  Hz, 1H), 4.52 (m, 1H), 4.37 (d,  $J = 11.6$  Hz, 1H), 4.12 (m, 1H), 3.37 (s, 1H), 2.81 (s, 1H), 2.04 – 1.93 (m, 1H), 1.61 – 1.47 (m, 4H), 1.42 (s, 11H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  188.29, 154.22, 136.61, 128.54, 128.35, 128.24, 81.46, 80.37, 72.96, 51.97, 39.51, 30.31, 29.69, 28.26, 24.82, 18.99; IR (neat) 2937, 2091, 1682, 1412, 1366, 1166  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{21}\text{H}_{28}\text{NO}_4^+$ : 358.2013, found 358.2026;  $[\alpha]_{\text{D}}^{23} +22$  ( $c$  1.0,  $\text{CHCl}_3$ ).



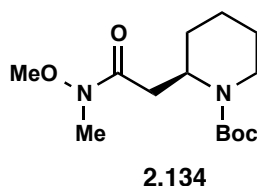
*(R)*-tert-Butyl 2-(2-Diazoacetyl)piperidine-1-carboxylate (**2.137**). *Warning: Large amounts of diazomethane were used for this transformation. Proper care should be taken when handling this highly explosive reagent. All glassware used was free of cracks, scratches or ground-glass joints and a blast shield was used. N-Boc-D-pipecolic acid (9.9 g, 43 mmol, 1.00 equiv) was taken into THF (100 mL) with stirring and cooled to 0*

°C with an ice bath. The reaction solution was treated with TEA (6.6 mL, 47 mmol, 1.10 equiv) and allowed to react for 15 min to fully deprotonate the carboxylic acid. With the addition of ethyl chloroformate (4.5 mL, 47 mmol, 1.10 equiv), a thick white precipitate formed. Stirring was continued for 15 min, and then stopped. In a separate flask, an ice-cold ethereal solution of diazomethane was prepared and, without stirring, was carefully decanted into the freshly prepared anhydride reaction flask using a glass funnel. The reaction solution was lightly stirred for 4 seconds, then stirring was stopped. The mixture was allowed to warm to rt and react overnight. Any additional Diazomethane was carefully quenched with 0.5 N acetic acid (100 mL). The dropwise addition of saturated NaHCO<sub>3</sub> (sat. *aq.*) regulated the solution back to a basic pH 8-9 with gentle stirring. The organic and aqueous layers were separated. The organic phase was washed twice each with saturated NaHCO<sub>3</sub> (sat. *aq.*) and brine, then dried over sodium sulfate. The solvent was evaporated under reduced pressure. The diazoketone was purified by SiO<sub>2</sub> flash column chromatography (15% EtOAc/hexanes) to provide 8.66 g (72%) of the title compound as a yellow oil; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.41 (s, 1H), 4.84 – 4.57 (m, 1H), 4.11-3.99 (m, 1H), 2.82 (s, 1H), 2.36 – 2.17 (m, 1H), 1.63-1.38 (m, 14H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 194.62, 155.10, 79.92, 53.30, 51.96, 28.33, 26.71, 24.78, 20.44;



2.138

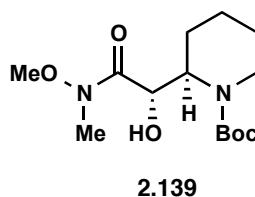
*(R)*-2-(1-(*tert*-Butoxycarbonyl)piperidin-2-yl)acetic Acid (**2.138**). Diazoketone **2.137** (8.66 g, 34.2 mmol, 1.00 equiv) was dissolved in 100 mL of a THF/H<sub>2</sub>O (1:1) solution under a N<sub>2</sub> atmosphere and protected from light. A solution of silver trifluoroacetate (1.51 g, 6.84 mmol, 0.20 equiv) in TEA (100 mL) was added dropwise and allowed to stir overnight. The volatiles were removed under reduced pressure. Sat. aq. NaHCO<sub>3</sub> was added and the resulting solution stirred for 1 h. The aqueous solution was extracted with Et<sub>2</sub>O, acidified with 10% HCl to a pH of 2, extracted with EtOAc (3x), dried (MgSO<sub>4</sub>) and concentrated under reduced pressure to give 8.1 g (97%) of the title compound as a pale yellow oil, which was used in the next step without further purification. The spectral data was identical to that reported in the literature.<sup>358</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.65 (s, 1H), 4.76 – 4.60 (m, 1H), 3.97 (d, *J* = 13.1 Hz, 1H), 2.75 (td, *J* = 14.1, 13.5, 2.7 Hz, 1H), 2.65 – 2.46 (m, 2H), 1.69 – 1.55 (m, 4H), 1.42 (s, 11H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 176.92, 154.92, 79.94, 47.76, 39.17, 35.15, 28.30, 28.25, 25.18, 18.77.



*(R)*-*tert*-Butyl 2-(2-(Methoxy(methyl)amino)-2-oxoethyl)piperidine-1-carboxylate (**2.134**).

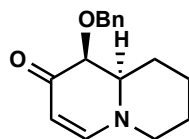
To a stirring solution of homopipercolic acid **2.138** (8.07 g, 33.2 mmol, 1.00 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) cooled to 0 °C were added *N,O*-dimethylhydroxylamine hydrochloride (3.57 g, 36.6 mmol, 1.10 equiv), NMM (3.70 g, 4.02 mL, 36.6 mmol, 1.10 equiv), and

EDCI (7.01 g, 36.6 mmol, 1.10 equiv) in sequence, and the reaction was allowed to warm to rt overnight. The reaction mixture was transferred to a separatory funnel containing 10% aq. HCl and CH<sub>2</sub>Cl<sub>2</sub>. The layers were separated and the aqueous layer was extracted twice more with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layers were washed with NaHCO<sub>3</sub> (aq.), the layers were separated, and the aqueous layers were extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layers were dried over MgSO<sub>4</sub>, concentrated, and purified by SiO<sub>2</sub> flash column chromatography (40% EtOAc/hexanes) to give 9.21 g (98%) of the title compound as a yellow oil. The spectral data are consistent with those reported in the literature.<sup>46</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.83 – 4.62 (m, 1H), 4.10 – 3.88 (m, 1H), 3.68 (s, 3H), 3.15 (s, 3H), 2.92 – 2.74 (m, 1H), 2.64 (qd, *J* = 14.0, 7.3 Hz, 2H), 1.68 – 1.51 (m, 6H), 1.43 (s, 9H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.27, 154.80, 79.32, 61.27, 47.70, 39.36, 32.81, 32.11, 28.42, 25.36, 18.99.



(*R*)-*tert*-Butyl 2-((*S*)-1-Hydroxy-2-(methoxy(methyl)amino)-2-oxoethyl)piperidine-1-carboxylate (**2.139**). THF (20 mL) was added to a flame dried vial and cooled to -78 °C under a N<sub>2</sub> atmosphere. NaHMDS (16.6 mL, 16.6 mmol, 2.50 equiv, 1.0M in THF) was then added. Weinreb amide **2.134** (1.91 g, 6.65 mmol, 1.00 equiv) in THF (10 mL) was added dropwise over 5 minutes. The reaction stirred at -78 °C for 30 minutes and oxaziridine **2.129** (4.58 g, 16.6 mmol, 2.50 equiv) in THF (10 mL) was added via

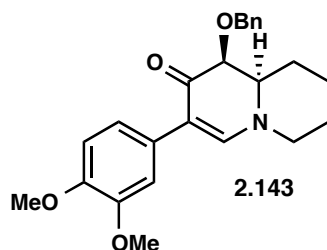
cannulation and stirred at  $-78$  for 1 hour. The reaction mixture was quenched with  $\text{NH}_4\text{Cl}$  and allowed to warm to rt. The organic layer was removed under reduced pressure and the mixture was diluted with  $\text{Et}_2\text{O}$  and the aqueous layer was separated and washed with  $\text{Et}_2\text{O}$  (3X). The combined organic layers were dried ( $\text{MgSO}_4$ ), filtered, concentrated under reduced pressure. The crude reaction mixture was purified via flash column chromatography (40% EtOAc/hexanes) to give 1.71 g (85%) of the  $\alpha$ -hydroxy amide as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.63 (t,  $J = 8.2$  Hz, 1H), 4.21 (s, 1H), 4.06 – 3.85 (m, 1H), 3.67 (s, 3H), 3.18 (s, 3H), 3.01 (d,  $J = 13.4$  Hz, 1H), 1.96 (d,  $J = 13.1$  Hz, 1H), 1.66 – 1.48 (m, 4H), 1.38 (s, 11H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.58, 154.76, 79.32, 71.79, 61.40, 32.47, 28.39, 28.37, 24.88, 24.77, 19.92, 19.69, 18.96; IR (neat) 3353, 2936, 1689, 1665, 1410, 1365, 1164; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_5^+$ : 303.1914, found 303.1910;  $[\alpha]_{\text{D}}^{23} +13$  ( $c$  1.0,  $\text{CHCl}_3$ ).



**2.140**

*(1S,9aR)-1-(Benzyloxy)-7,8,9,9a-tetrahydro-1H-quinolizin-2(6H)-one* (**2.140**). Ynone **2.135** (1.32 g, 3.37 mmol, 1.00 equiv) was dissolved in formic acid to a concentration of 1M. Sodium iodide (1.52 g, 10.1 mmol, 3.00 equiv) was added and that was allowed to stir for 24 h. Formic acid was evaporated by passing  $\text{N}_2$  over the solution. The formate salt was precipitated out with  $\text{Et}_2\text{O}$ /hexanes (1:1).  $\text{K}_2\text{CO}_3$  (2.60 g, 18.8 mmol, 5.00 equiv) was slurried in 50 mL MeOH. The salt from step one was dissolved in a minimal amount

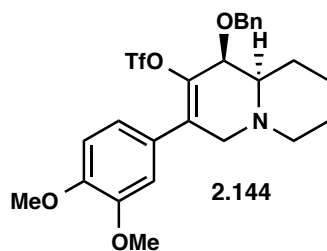
of MeOH and added to the K<sub>2</sub>CO<sub>3</sub> slurry slowly over 1.5 hours via an addition funnel. Upon completion, monitored via TLC (1.5 h), the MeOH was removed via rotary evaporation. The residue was dissolved in H<sub>2</sub>O and that was extracted with EtOAc (3x), dried with MgSO<sub>4</sub>, and concentrated under reduced pressure. The crude material was purified via SiO<sub>2</sub> flash column chromatography (100% EtOAc) to give 0.642 g (74%) of the title compound as a brown oil: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.43 – 7.21 (m, 5H), 6.85 (d, *J* = 7.5 Hz, 1H), 5.05 – 4.92 (m, 2H), 4.61 (d, *J* = 11.6 Hz, 1H), 3.58 (d, *J* = 8.2 Hz, 1H), 3.45 – 3.27 (m, 2H), 3.05 (td, *J* = 12.7, 3.0 Hz, 1H), 1.98 – 1.81 (m, 2H), 1.76 – 1.64 (m, 1H), 1.57 – 1.33 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 191.18, 153.01, 137.99, 128.50, 128.39, 128.36, 128.32, 127.79, 127.74, 96.88, 79.81, 73.16, 62.46, 54.00, 28.09, 26.29, 23.50; IR (neat) 2938, 1634, 1587, 1446, 1389, 1324, 1237, 1143 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>16</sub>H<sub>20</sub>NO<sub>2</sub><sup>+</sup>: 258.1489, found 258.11487; [α]<sub>D</sub><sup>23</sup> +150 (*c* 1.0, CHCl<sub>3</sub>).



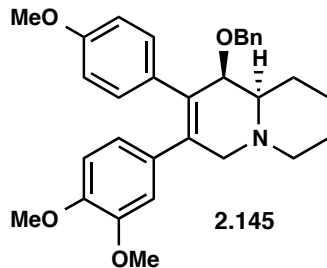
(1*S*,9*aR*)-1-(Benzyloxy)-3-(3,4-dimethoxyphenyl)-7,8,9,9*a*-tetrahydro-1*H*-quinolizin-2(6*H*)-one (**2.143**). Enaminone **2.140** (0.055 g, 0.22 mmol, 1.00 equiv), [Pd(OAc)<sub>2</sub>]<sub>3</sub> (0.0145 g, 0.022 mmol, 0.1 equiv), anhydrous Cu(OAc)<sub>2</sub> (0.12 g, 0.65 mmol, 3.00 equiv), and K<sub>2</sub>CO<sub>3</sub> (0.060 g, 0.43 mmol, 2.00 equiv) were combined in a *t*BuOH/AcOH/DMSO



solution (20:5:1, 2 mL) and stirred for 5 min. The reaction mixture was heated to 60 °C and potassium 3,4-dimethoxyphenyltrifluoroborate (0.21g, 0.86 mmol, 4.00 equiv) in acetone/H<sub>2</sub>O (2:1, 9 mL) was added to the reaction mixture over 2 h. An additional portion of [Pd(OAc)<sub>2</sub>]<sub>3</sub> (0.0070 g, 0.011 mmol) was added to the stirring mixture and stirred for an additional 2 h. The reaction mixture was then cooled rt and K<sub>2</sub>CO<sub>3</sub> was added to the dark reaction medium until gas evolution ceased. The basic solution was filtered through a celite plug eluting with EtOAc, concentrated, and purified by SiO<sub>2</sub> flash column chromatography (80% EtOAc/hexanes) to give 0.067 g (90%) of the title compound as a yellow oil: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.37 – 7.31 (m, 5H), 7.12 (s, 1H), 7.09 (dd, *J* = 4.0, 2.0 Hz, 1H), 6.88 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.84 – 6.80 (m, 1H), 5.16 (d, *J* = 7.1 Hz, 1H), 4.97 (d, *J* = 7.0 Hz, 1H), 4.08 (d, *J* = 9.6 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.56 – 3.45 (m, 1H), 3.29 (ddd, *J* = 11.4, 9.5, 3.0 Hz, 1H), 3.12 (td, *J* = 12.7, 3.2 Hz, 1H), 2.05 – 1.96 (m, 1H), 1.72 – 1.41 (m, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 188.52, 152.22, 148.67, 147.49, 138.11, 128.66, 128.53, 127.91, 127.80, 119.55, 119.41, 111.83, 111.29, 95.18, 70.59, 61.91, 56.10, 55.99, 54.19, 28.65, 26.21, 23.33; IR (neat) 2936, 1634, 1595, 1515, 1251, 1028 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>24</sub>H<sub>28</sub>NO<sub>4</sub><sup>+</sup>: 394.2013, found 394.2010; [α]<sub>D</sub><sup>23</sup> +11 (*c* 1.0, CHCl<sub>3</sub>).

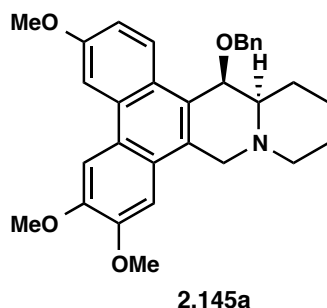


(1*S*,9*aR*)-1-(Benzyloxy)-3-(3,4-dimethoxyphenyl)-4,6,7,8,9,9*a*-hexahydro-1*H*-quinolizin-2-yl trifluoromethanesulfonate (**2.144**). Quinolizidone **2.143** (0.061 g, 0.15 mmol, 1.00 equiv) was dissolved in THF (1 mL) and cooled to  $-78\text{ }^{\circ}\text{C}$ . L-Selectride (1.0 M in THF, 0.17 mL, 0.17 mmol, 1.10 equiv) was added dropwise to the stirring solution and the reaction was maintained at  $-78\text{ }^{\circ}\text{C}$  for 30 min at which time the solution was warmed to rt. Comins' reagent (0.067 g, 0.17 mmol, 1.10 equiv) was subsequently added to the reaction mixture and allowed to stir at rt overnight. The resulting solution was quenched with saturated  $\text{NaHCO}_3$  (aq.) (1.0 mL), diluted with  $\text{CH}_2\text{Cl}_2$  (3.0 mL), dried with  $\text{K}_2\text{CO}_3$ , and filtered through celite. The resulting filtrate was concentrated and purified via  $\text{SiO}_2$  flash column chromatography (1:1 EtOAc/hexanes with 1% TEA) to give 0.036 g (46%) of the title compound as a yellow oil:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.38 – 7.32 (m, 5H), 7.08 (dd,  $J = 3.9, 2.0$  Hz, 1H), 6.91 – 6.85 (m, 1H), 6.85 – 6.79 (m, 1H), 5.16 (d,  $J = 7.1$  Hz, 1H), 4.97 (d,  $J = 7.1$  Hz, 1H), 4.08 (d,  $J = 9.6$  Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.60 – 3.36 (m, 2H), 3.29 (ddd,  $J = 11.3, 9.5, 3.0$  Hz, 1H), 3.12 (td,  $J = 12.7, 3.2$  Hz, 1H), 2.90 – 2.72 (m, 1H), 1.94 (dq,  $J = 11.9, 2.6$  Hz, 1H), 1.68 – 1.42 (m, 5H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  149.48, 148.90, 140.79, 138.02, 128.61, 128.51, 128.37, 128.26, 128.11, 127.83, 121.37, 120.46, 111.25, 111.09, 75.67, 73.44, 63.16, 56.08, 55.99, 55.48, 27.29, 25.36, 23.87; IR (neat) 2936, 1519, 1414, 1211, 1141, 1031  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{25}\text{H}_{29}\text{F}_3\text{NO}_6\text{S}^+$ : 528.1662, found 528.1660;  $[\alpha]_{\text{D}}^{23} -52$  ( $c$  1.0,  $\text{CHCl}_3$ ).



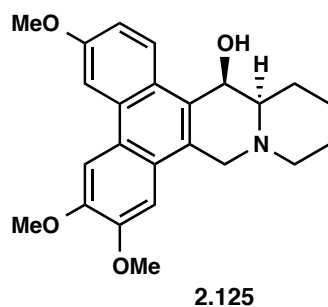
(9*R*,9*aR*)-9-(Benzyloxy)-7-(3,4-dimethoxyphenyl)-8-(4-methoxyphenyl)-2,3,4,6,9,9*a*-hexahydro-1*H*-quinolizine (**2.145**). A reaction vessel containing ZnBr<sub>2</sub> (0.028 g, 0.12 mmol, 3.50 equiv) was flame-dried under vacuum and upon reaching rt it was released from vacuum and fitted immediately with a septum and placed under a nitrogen atmosphere. THF (1 mL) was added to the purged reaction vessel and once homogeneity was achieved, 4-methoxyphenylmagnesium bromide (0.5 M in THF, 0.21 mL, 0.11 mmol, 3 equiv) was added, resulting in a white slurry that was stirred for 10 min. This solution was transferred to a reaction vessel containing triflate **2.144** (0.020 g, 0.040 mmol, 1.00 equiv) and Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0040 g, 0.0040 mmol, 0.10 equiv) and the reaction mixture was heated to 60 °C. Monitoring by TLC (1:1 EtOAc/hexanes with 1% TEA) showed complete consumption of starting material within 1 h. The reaction was allowed to cool to rt and immediately purified via SiO<sub>2</sub> flash column chromatography to yield 0.035g (59%) of the title compound as a yellow oil; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.44 – 7.32 (m, 5H), 7.22 – 6.98 (m, 3H), 6.97 – 6.81 (m, 4H), 4.90 (d, *J* = 10.5 Hz, 1H), 4.65 (d, *J* = 10.5 Hz, 1H), 4.24 (ddd, *J* = 7.9, 3.2, 1.5 Hz, 1H), 3.95 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.75 (s, 1H), 3.60 – 3.53 (m, 1H), 3.12 – 2.95 (m, 2H), 2.43 (ddd, *J* = 10.7, 7.7,

3.2 Hz, 1H), 2.18 (qd,  $J = 11.4, 3.3$  Hz, 2H), 1.83 (dt,  $J = 12.5, 3.6$  Hz, 1H), 1.73 – 1.58 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  149.46, 148.76, 140.14, 137.63, 132.02, 128.47, 128.42, 128.12, 127.98, 125.38, 121.24, 119.12, 116.10, 114.75, 111.92, 111.49, 110.89, 110.41, 79.41, 73.48, 63.46, 59.01, 56.00, 55.87, 54.77, 30.22, 24.93, 23.24; IR (neat) 2930, 1606, 1511, 1246, 1030, 756  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{31}\text{H}_{36}\text{NO}_4^+$ : 486.2639, found 486.2645;  $[\alpha]_{\text{D}}^{23} -150$  ( $c$  1.0,  $\text{CHCl}_3$ ).



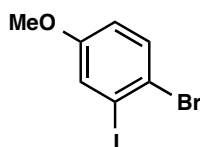
*(14aR,15R)-15-(Benzyloxy)-3,6,7-trimethoxy-11,12,13,14,14a,15-hexahydro-9H-dibenzo[f,h]pyrido[1,2-b]isoquinoline (2.145a)*. Quinolizidine **2.145** (0.023 g, 0.048 mmol, 1.00 equiv) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was cooled to  $-78$  °C under a nitrogen atmosphere.  $\text{VOF}_3$  (0.012 g, 0.097 mmol, 2.10 equiv) in a solution of TFA/ $\text{CH}_2\text{Cl}_2$ /EtOAc (1:1:1, 1 mL) was subsequently added to the stirring solution in one portion at  $-78$  °C. The reaction was monitored by TLC (1:1 EtOAc/hexanes with 1% TEA) and complete consumption of starting material was observed within 1 h. The reaction was quenched with 10% aq. NaOH (10 mL) and the solution was warmed to rt. The layers were separated and the aqueous phase was extracted  $\text{CH}_2\text{Cl}_2$  (2x). The combined organic extracts were dried with  $\text{MgSO}_4$ , concentrated, and purified via  $\text{SiO}_2$  flash column

chromatography to give 0.009 g (39%) of the title compound as an off white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.44 (d,  $J = 9.2$  Hz, 1H), 7.95 (s, 1H), 7.90 (d,  $J = 2.5$  Hz, 1H), 7.27 – 7.18 (m, 5H), 7.14 – 7.07 (m, 2H), 5.40 (d,  $J = 7.7$  Hz, 1H), 4.33 – 4.19 (m, 2H), 4.12 (s, 3H), 4.07 (s, 3H), 4.02 (s, 3H), 3.91 (d,  $J = 10.9$  Hz, 1H), 3.81 – 3.76 (m, 1H), 3.74 – 3.60 (m, 1H), 3.27 (d,  $J = 11.1$  Hz, 1H), 2.69 (ddd,  $J = 10.6, 7.7, 2.9$  Hz, 1H), 2.59 – 2.35 (m, 2H), 2.02 – 1.90 (m, 1H), 1.78 (t,  $J = 5.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  157.82, 149.59, 149.11, 138.95, 130.82, 129.06, 128.31, 127.82, 127.48, 127.37, 125.16, 124.73, 124.64, 124.58, 115.07, 104.57, 104.10, 103.79, 78.40, 65.34, 60.86, 56.46, 56.15, 55.59, 55.55, 31.52, 25.34, 24.25; IR (neat) 2928, 2253, 1611, 1511, 1470, 1256, 1204, 1140, 1040  $\text{cm}^{-1}$ ; MP = decomp.  $>214$   $^\circ\text{C}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+ \text{C}_{32}\text{H}_{34}\text{NO}_4^+$ : 484.2482, found 484.2484;  $[\alpha]_{\text{D}}^{23} -73$  ( $c$  1.0,  $\text{CHCl}_3$ ).



*15-Hydroxyboehmeriasin A* (**2.125**). Quinolizidine **2.145a** (0.007 g, 0.02 mmol, 1.00 equiv) and Pd/C (0.005 g) were suspended in MeOH and placed under a  $\text{H}_2$  atmosphere. The suspension was allowed to stir until completion (48 h), at which time was diluted with MeOH. The suspension was filtered, concentrated, and purified via  $\text{SiO}_2$  flash column chromatography (5% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give 5.5 mg (88%) of the title compound as white solid:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.43 (d,  $J = 9.1$  Hz, 1H), 7.94 (s, 1H), 7.90

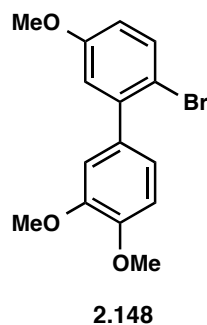
(d,  $J = 2.6$  Hz, 1H), 7.13 – 7.06 (m, 2H), 5.39 (d,  $J = 7.7$  Hz, 1H), 4.21 (d,  $J = 11.3$  Hz, 1H), 4.12 (s, 3H), 4.05 (s, 3H), 4.02 (s, 3H), 3.21 (d,  $J = 11.1$  Hz, 1H), 2.74 – 2.60 (m, 1H), 2.44 (ddt,  $J = 26.6, 11.2, 4.5$  Hz, 2H), 2.30 – 2.18 (m, 1H), 1.98 – 1.89 (m, 2H), 1.69 – 1.51 (m, 5H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  157.84, 149.59, 149.12, 130.83, 127.49, 127.39, 125.16, 124.71, 124.62, 124.58, 115.08, 104.60, 104.10, 103.78, 65.34, 60.84, 56.15, 55.61, 55.56, 55.52, 55.49, 29.85, 25.32, 24.25; IR (neat) 3151, 2988, 2900, 1532, 1390, 1237, 1149, 1034, 783  $\text{cm}^{-1}$ ; HRMS (ESI+)  $m/e$  calc'd for  $[\text{M}+\text{H}]^+$   $\text{C}_{24}\text{H}_{28}\text{NO}_4^+$ : 394.2013, found 394.2014;  $[\alpha]_{\text{D}}^{23}$   $-92$  ( $c$  0.50,  $\text{CHCl}_3$ ).



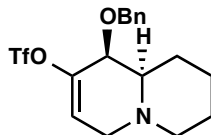
**2.149**

*1-Bromo-2-iodo-4-methoxybenzene* (**2.149**). Bromine (4.90 mL, 95.6 mmol, 1.10 equiv) was added dropwise to a solution of 3-iodoanisole (20.2 g, 86.3 mmol, 1.00 equiv) in glacial acetic acid (130 mL) and the resulting orange mixture was stirred at ambient temperature for 24 h. The resulting solution was diluted with water (250 mL) and extracted with hexanes (3x). The combined orange extracts were washed with aq.  $\text{Na}_2\text{S}_2\text{O}_3$ , brine, dried ( $\text{MgSO}_4$ ), and concentrated to an orange oil. The crude oil was purified via  $\text{SiO}_2$  flash column chromatography (15% EtOAc/hexanes) to give 22.7 g (84%) as a pale brown oil. The spectral data was identical to that reported in the literature.<sup>277</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.47 (d,  $J = 8.8$  Hz, 1H), 7.38 (d,  $J = 2.9$  Hz, 1H), 6.77 (dd,  $J = 8.8, 2.9$  Hz, 1H), 3.77 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  158.69,

132.62, 125.38, 120.25, 116.02, 101.06, 55.67.



*2-Bromo-3',4',5-trimethoxy-1,1'-biphenyl* (**2.148**). DME (380 mL) and Na<sub>2</sub>CO<sub>3</sub> (137 mL, 274 mmol, 5.00 equiv, 2.0 M *aq.* solution) were added to a round-bottomed flask and degassed under reduced pressure for 30 minutes. Iodobromoveratrole **2.149** (17.2 g, 54.9 mmol, 1.00 equiv), 3,4-dimethoxyphenylboronic acid (13.0 g, 71.3 mmol, 1.3 equiv), LiCl (7.0 g, 166 mmol, 3.0 equiv) and Pd(dppf)Cl<sub>2</sub> (2.24 g, 2.75 mmol, 0.05 equiv) were added all at once and the reaction mixture was heated to 80 °C under a N<sub>2</sub> atmosphere. After 16 h the reaction was quenched with a saturated solution of NH<sub>4</sub>Cl (*aq.*) and added to a separatory funnel. The product was extracted with EtOAc (3x). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The title compound was obtained as a colorless oil (12.8 g, 72% yield) after SiO<sub>2</sub> flash column chromatography (20% EtOAc/hexanes). The spectral data was identical to that reported in the literature.<sup>277</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.53 (d, *J* = 8.8 Hz, 1H), 7.00 – 6.92 (m, 3H), 6.89 (d, *J* = 3.0 Hz, 1H), 6.76 (dd, *J* = 8.8, 3.1 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.81 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 158.77, 148.58, 148.24, 143.10, 133.81, 133.70, 121.58, 116.82, 114.39, 113.28, 112.82, 110.60, 55.96, 55.88, 55.54.

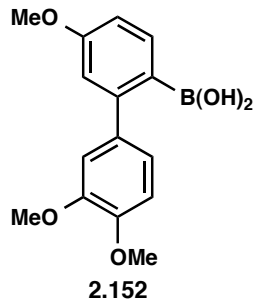


**2.150**

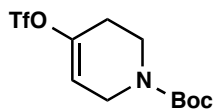
*(1S,9aR)-1-(Benzyloxy)-4,6,7,8,9,9a-hexahydro-1H-quinolizin-2-yl-trifluoromethane*

*Sulfonate* (**2.150**). Enaminone **2.140** (0.479 g, 1.86 mmol, 1.00 equiv) was dissolved in anhydrous THF (10 mL) under a N<sub>2</sub> atmosphere. The solution was cooled to –78 °C at which point L-Selectride (2.05 mL, 2.05 mmol, 1.10 equiv, 1.0 M in THF) was added over 15 minutes. After stirring for 1 h, the reaction was slowly warmed to 0 °C over 2 hours. The reaction mixture was once again cooled to –78 °C and Comins' reagent (0.803 g, 2.05 mmol, 1.10 equiv) was added all at once. The mixture was stirred for another hour at –78 °C and then slowly warmed to 0 °C over 2 hours. The reaction was quenched with a saturated solution of NaHCO<sub>3</sub> (aq.) and added to a separatory funnel. The product was extracted with EtOAc (3x). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The title compound was obtained as a colorless oil (0.53 g, 73% yield) after SiO<sub>2</sub> flash column chromatography (20% EtOAc/hexanes with 1% TEA): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.36 – 7.28 (m, 5H), 5.87 (dd, *J* = 5.1, 1.5 Hz, 1H), 4.84 (d, *J* = 10.6 Hz, 1H), 4.53 (d, *J* = 10.7 Hz, 1H), 3.95 (dd, *J* = 5.8, 1.9 Hz, 1H), 3.30 (ddd, *J* = 17.1, 5.4, 1.7 Hz, 1H), 2.99 – 2.84 (m, 2H), 2.33 – 2.21 (m, 1H), 2.10 (qd, *J* = 7.3, 6.3, 4.5 Hz, 2H), 2.00 – 1.46 (m, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 146.27, 137.45, 128.52, 128.38, 128.37, 128.11, 127.91, 117.92, 78.21, 74.68, 63.25, 55.00, 52.86, 29.99, 25.03, 23.29; IR (neat) 2794, 1689, 1419, 1210, 1143, 1024, 879 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>17</sub>H<sub>21</sub>F<sub>3</sub>NO<sub>4</sub>S<sup>+</sup>: 392.1138, found 392.1146; [α]<sub>D</sub><sup>23</sup> –83 (*c* 1.0, CHCl<sub>3</sub>).



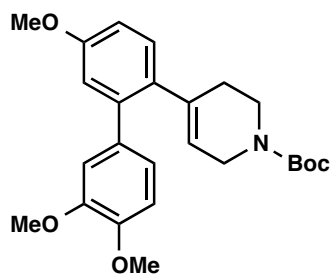


(3',4',5-Trimethoxy-[1,1'-biphenyl]-2-yl)boronic acid (**2.152**). Biaryl bromide **2.148** (4.98 g, 15.4 mmol, 1.00 equiv) was dissolved in anhydrous THF (20 mL) and cooled to  $-78$  °C. *n*-BuLi (6.80 mL, 17.0 mmol, 1.10 equiv, 2.5 M solution in hexanes) was added dropwise and allowed to stir for 30 minutes. Triisopropyl borate (7.54 g, 9.22 mL, 40.1 mmol, 2.60 equiv) was added dropwise and allowed to stir for 4 hours eventually warming to rt. The resulting boronic ester was hydrolyzed by stirring in 10% HCl (50 mL) for 2 h. The solution was extracted with EtOAc (3x). The combined organic extracts were dried ( $\text{MgSO}_4$ ), concentrated under reduced pressure, and purified via  $\text{SiO}_2$  flash column chromatography (100 % EtOAc). The resulting oil was dissolved in a minimal amount of EtOAc and crystallized out by the addition of hexanes to give 1.72 g (39%) of the title compound as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.99 – 6.84 (m, 3H), 6.84 – 6.75 (m, 2H), 6.68 (dd,  $J = 8.4, 2.5$  Hz, 1H), 3.95 (s, 3H), 3.85 (d,  $J = 1.5$  Hz, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  161.99, 151.86, 148.37, 139.78, 137.03, 121.35, 115.95, 112.77, 112.25, 111.97, 111.25, 110.57, 56.05, 55.89, 55.18.



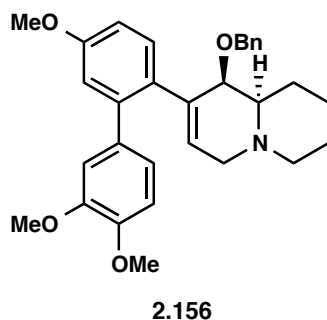
2.154

*tert*-Butyl 4-(((Trifluoromethyl)sulfonyl)oxy)-5,6-dihydropyridine-1(2*H*)-carboxylate (2.154). *N*-Boc-piperidone (0.51 g, 2.51 mmol, 1.00 equiv) and Comins' reagent (1.18 g, 3.01 mmol, 1.20 equiv) were dissolved in anhydrous THF (10 mL) and cooled to  $-78\text{ }^{\circ}\text{C}$ . LiHMDS (2.76 mL, 2.76 mmol, 1.10 equiv, 1 M in THF) was added dropwise. The solution stirred overnight eventually warming to rt. The reaction was quenched with  $\text{NH}_4\text{Cl}$  (aq.) and extracted with EtOAc (3x). The combined organic extracts were dried ( $\text{MgSO}_4$ ), concentrated under reduced pressure, and purified via  $\text{SiO}_2$  flash column chromatography (25% EtOAc/hexanes) to give 0.519 g (62%) of the title compound as a clear oil. The spectral data is identical to that reported in the literature.<sup>359</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.76 (s, 1H), 4.03 (q,  $J = 3.0$  Hz, 2H), 3.62 (t,  $J = 5.7$  Hz, 2H), 2.52 – 2.32 (m, 2H), 1.46 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  154.31, 149.48, 139.44, 80.60, 60.49, 41.93, 28.40, 28.31, 21.24.



2.155

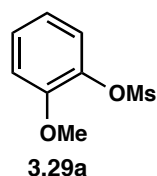
*tert*-Butyl-4-(3',4',5-Trimethoxy-[1,1'-biphenyl]-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (**2.155**). See **2.148** for procedure. Triflate **2.154** (0.17 mmol) yielded 0.058 g (79%) of the title compound as a yellow oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.32 (t, *J* = 8.3 Hz, 2H), 7.17 (dd, *J* = 6.2, 2.7 Hz, 3H), 6.99 (dd, *J* = 8.2, 2.0 Hz, 1H), 5.43 (d, *J* = 5.1 Hz, 1H), 4.26 (d, *J* = 10.4 Hz, 1H), 4.17 (d, *J* = 10.3 Hz, 1H), 3.95 (s, 3H), 3.88 (s, 3H), 3.69 (s, 3H), 3.17 (d, *J* = 16.7 Hz, 1H), 2.87 (d, *J* = 11.6 Hz, 1H), 2.64 (d, *J* = 17.1 Hz, 1H), 2.11 – 2.06 (m, 1H), 1.48 (s, 9H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 158.66, 148.22, 147.92, 141.95, 138.55, 134.66, 130.67, 128.50, 128.17, 128.05, 127.86, 127.50, 127.20, 121.35, 115.53, 113.55, 112.26, 110.72, 81.32, 74.59, 63.67, 60.51, 56.05, 55.93, 55.67, 55.49, 54.69, 30.42, 25.26, 24.12.



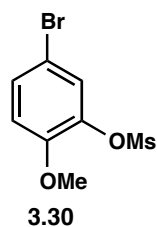
(9*R*,9*aR*)-9-(Benzyloxy)-8-(3',4',5-trimethoxy-[1,1'-biphenyl]-2-yl)-2,3,4,6,9,9*a*-hexahydro-1*H*-quinolizine (**2.156**). See **2.148** for procedure. Triflate **2.150** (0.18 mmol) yielded 0.071 g (83%) of the title compound as a yellow oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.29 (d, *J* = 8.4 Hz, 1H), 7.19 – 7.14 (m, 3H), 6.92 – 6.77 (m, 7H), 5.47 – 5.37 (m, 1H), 4.26 (d, *J* = 10.3 Hz, 1H), 4.17 (d, *J* = 10.4 Hz, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H), 3.69 (s, 3H), 3.14 (ddd, *J* = 16.9, 5.1, 1.6 Hz, 1H), 2.86 (dd, *J* = 10.3, 2.5 Hz, 1H),

2.62 (dt,  $J = 16.8, 2.6$  Hz, 1H), 2.14 (td,  $J = 7.3, 3.7$  Hz, 1H), 2.11 – 2.04 (m, 2H), 1.74 (d,  $J = 4.2$  Hz, 1H), 1.60 (d,  $J = 7.7$  Hz, 2H);  $[\alpha]_{\text{D}}^{23} -140$  ( $c$  1.0,  $\text{CHCl}_3$ ).

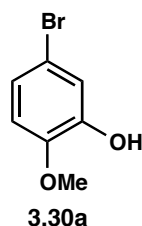
#### 4.4 Chapter 3



*2-Methoxyphenyl Methanesulfonate (3.29a)*: Guaiacol (**3.29**) (11.2 g, 10.0 mL, 90.2 mmol 1.00 equiv) was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (90 mL) and cooled to 0 °C. TEA (18.9 mL, 135 mmol, 1.50 equiv) was added via a steady stream. Methanesulfonyl chloride (12.9 g, 8.72 mL, 113 mmol, 1.25 equiv) was added over 15 minutes and allowed to stir for an additional 15 minutes. The reaction was quenched with  $\text{H}_2\text{O}$  and the phases were separated. The aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$  (2x) and the combined organic phases were washed with brine, dried ( $\text{MgSO}_4$ ) and concentrated under reduce pressure to yield 17.9 g (98%) of the title compound as a colorless oil. The crude reaction mixture was carried forward without purification. The spectral data was identical to that reported in the literature.<sup>336</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.27 – 7.20 (m, 2H), 6.98 (dd,  $J = 8.1, 1.5$  Hz, 1H), 6.92 (td,  $J = 7.8, 1.4$  Hz, 1H), 3.83 (s, 3H), 3.11 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  151.44, 138.33, 128.34, 124.40, 121.00, 113.02, 55.91, 38.15.

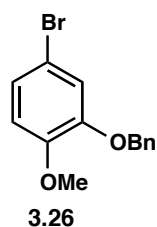


*5-Bromo-2-methoxyphenyl methanesulfonate (3.30)*. Mesylate **3.29a** (17.9 g, 88.5 mmol, 1.00 equiv) was dissolved in DMF (250 mL). A solution of NBS (20.5 g, 115 mmol, 1.30 equiv) in DMF (100 mL) was added dropwise as allowed to stir for 24 h. The reaction was quenched with H<sub>2</sub>O and extracted with Et<sub>2</sub>O (3x). The combined organic extracts were washed with H<sub>2</sub>O, brine, dried (MgSO<sub>4</sub>), and concentrated under reduced pressure to yield 23.9 g (96%) of the title compound as a yellow solid which was carried forward without further purification. The spectral data was identical to that reported in the literature.<sup>336</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.45 (d, *J* = 2.4 Hz, 1H), 7.39 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 3.88 (s, 3H), 3.20 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 150.89, 138.58, 131.06, 127.61, 114.15, 112.33, 56.26, 38.52.



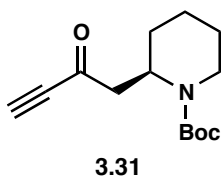
*5-Bromo-2-methoxyphenol (3.30a)*. Aryl bromomesylate **3.30** (23.9 g, 85.0 mmol 1.00 equiv) was dissolved in THF (50 mL). 6N NaOH (*aq.*) (300 mL) was added and heated at reflux for 5 h. The reaction was allowed to cool to rt and extracted with Et<sub>2</sub>O (3x). The

combined organic extracts were washed with H<sub>2</sub>O, brine, dried (MgSO<sub>4</sub>), and concentrated under reduced pressure to give 17.1 g (99%) of the title compound as a yellow oil. The crude material was carried forward without purification. The spectral data was identical to that reported in the literature.<sup>336</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.07 (dd, *J* = 2.4, 0.7 Hz, 1H), 6.96 (ddd, *J* = 8.5, 2.4, 0.7 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 3.86 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 146.32, 145.69, 122.64, 117.71, 114.15, 111.74, 56.06.

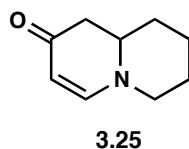


*2-(Benzyloxy)-4-bromo-1-methoxybenzene* (**3.26**). Phenol **3.30a** (17.1 g, 84.2 mmol, 1.00 equiv) and K<sub>2</sub>CO<sub>3</sub> (58.1 g, 420 mmol, 5 equiv) were suspended in DMF (150 mL). Benzyl Bromide (28.7 g, 20.0 mL, 168 mmol, 2 equiv) was added via a steady stream. Upon completion (monitored via TLC, 5 h) the reaction was quenched with H<sub>2</sub>O and extracted with Et<sub>2</sub>O (3x). the combined organic extracts were washed with H<sub>2</sub>O, brine, dried (MgSO<sub>4</sub>), concentrated under reduced pressure, and purified via recrystallization (EtOAc/hexanes) to give 21.2 g (85%) of the title compound as a white solid. The spectral data was identical to that reported in the literature.<sup>360</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.49 – 7.29 (m, 5H), 7.04 (d, *J* = 8.1 Hz, 2H), 6.76 (d, *J* = 8.2 Hz, 1H), 5.11 (s, 2H), 3.86 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 149.02, 148.96, 136.41, 128.62,

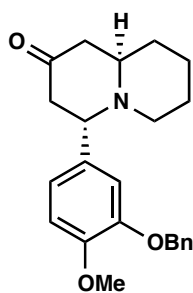
128.06, 127.38, 123.98, 117.28, 113.08, 112.53, 71.21, 56.16.



(±)-*tert*-Butyl 2-(2-Oxobut-3-yn-1-yl)piperidine-1-carboxylate (**3.31**). (±)-Weinreb amide **2.134** (5.66 g, 19.8 mmol, 1.00 equiv) was dissolved in THF (20 mL) and cooled to 0 °C. Ethynylmagnesium bromide (198 mL, 99.0 mmol, 0.5 M in THF, 5.00 equiv) was added dropwise. Upon completion (monitored via TLC, 5 h) the reaction was quenched with NH<sub>4</sub>Cl (aq.) and extracted with Et<sub>2</sub>O (3x). The combined organic extracts were washed with water, brine, dried (MgSO<sub>4</sub>), concentrated under reduced pressure, and purified via SiO<sub>2</sub> flash column chromatography (40% EtOAc/hexanes) to yield 4.53 g (91%) of the title compound as a white solid. The spectral data was identical to that reported in the literature.<sup>46</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.83 (bs, 1H), 4.01 (d, *J* = 11.0 Hz, 1H), 3.25 (s, 1H), 2.86 (dd, *J* = 14.4, 7.0 Hz, 1H), 2.82-2.72 (m, 2H), 1.75-1.60 (m, 4H), 1.57-1.47 (m, 2H), 1.45 (s, 9H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 185.25, 154.75, 81.70, 80.04, 79.01, 47.59, 46.03, 39.46, 28.63, 28.50, 25.35, 19.03.



(±)-7,8,9,9a-Tetrahydro-1H-quinolizin-2(6H)-one (**3.25**). See **1.42** section 4.2 for procedure. Ynone **3.31** (4.53 g, 18.0 mmol) yielded 2.50 g (91%) of the title compound as a white solid. The spectral data was identical to that reported in the literature.<sup>46</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.85 (d, *J* = 7.6 Hz, 1H), 4.96 (d, *J* = 7.6 Hz, 1H), 3.50 – 3.18 (m, 2H), 2.97 (td, *J* = 12.7, 3.0 Hz, 1H), 2.46 (dd, *J* = 16.4, 5.6 Hz, 1H), 2.35 (dd, *J* = 16.4, 13.0 Hz, 1H), 1.93 – 1.67 (m, 3H), 1.67 – 1.29 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 192.48, 154.92, 99.57, 57.30, 53.12, 43.38, 31.82, 25.75, 23.31.

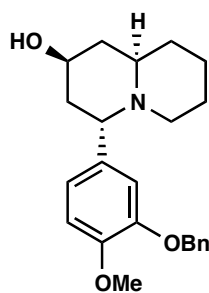


**3.33**

(±)-4-(3-(Benzyloxy)-4-methoxyphenyl)hexahydro-1H-quinolizin-2(6H)-one (**3.33**). Aryl bromide **3.26** (2.65 g, 9.03 mmol, 4.00 equiv) was dissolved in anhydrous THF and cooled to  $-78$  °C. *n*-BuLi (5.50 mL, 13.5 mmol, 6.00 equiv, 2.5 M in hexanes) was added dropwise over 20 minutes and allowed to stir for an additional 1 h. CuBr•DMS (0.928 g, 4.51 mmol, 2.00 equiv) in THF (4 mL) was added dropwise and warmed to 0 °C and allowed to stir for 2 h at that temperature. The mixture was re-cooled to  $-78$  °C and chlorotrimethylsilane (3.92 g, 4.57 mL, 36.1 mmol, 16.00 equiv) was added dropwise and allowed to stir for 30 minutes. Enaminone **3.25** (0.342 g, 2.26 mmol, 1.00 equiv) in THF (4 mL) was added dropwise and allowed to stir overnight eventually warming to rt.



NH<sub>4</sub>OH (5 mL) was added and the solution stirred at rt until it turned blue. The solution was acidified with concentrated HCl and stirred for 25 minutes. The solution was then made basic by the addition of NaHCO<sub>3</sub> (sat. *aq.*). After extraction with CH<sub>2</sub>Cl<sub>2</sub>, the combined organic layers were dried (MgSO<sub>4</sub>), concentrated under reduced pressure, and purified via SiO<sub>2</sub> flash column chromatography (1:1 EtOAc/hexanes with 1% TEA) to yield 0.438 g (53%) of the title compound as a yellow oil: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.28 – 7.20 (m, 5H), 6.71 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.47 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.39 (d, *J* = 2.1 Hz, 1H), 5.00 (s, 2H), 3.93 (dd, *J* = 6.7, 3.2 Hz, 1H), 3.81 (s, 3H), 2.79 – 2.69 (m, 1H), 2.44 – 2.30 (m, 4H), 2.23 – 2.01 (m, 3H), 1.81 (ddd, *J* = 10.4, 8.2, 5.2 Hz, 1H), 1.64 – 1.43 (m, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 209.64, 149.66, 146.79, 142.08, 128.39, 127.42, 126.57, 121.15, 118.25, 111.48, 81.66, 63.99, 56.16, 53.24, 51.32, 47.65, 46.24, 38.07, 32.68, 28.01, 24.65, 22.67, 14.12; IR (neat) 2944, 1715, 1510, 1261 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>23</sub>H<sub>28</sub>NO<sub>3</sub><sup>+</sup>: 366.2064, found 366.2068.

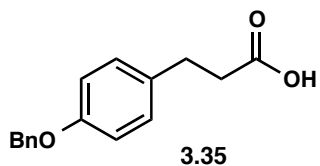


3.34

(±)-4-(3-(Benzyloxy)-4-methoxyphenyl)octahydro-1H-quinolizin-2-ol (3.34).

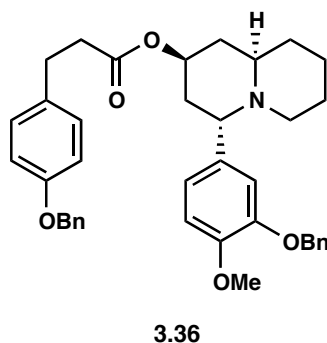
Quinolizidine **3.33** (0.414 g, 1.50 mmol, 1.00 equiv) was dissolved in anhydrous THF (5 mL) and cooled to -78 °C. L-Selectride (1.80 mL, 1.80 mmol, 1.20 equiv) was added

dropwise. Upon completion (1.5 h), the reaction was quenched with NH<sub>4</sub>Cl and allowed to warm to rt. The reaction was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x) and the combined organic extracts were concentrated under reduced pressure. The crude boronate residue was dissolved in EtOH (10 mL) and heated at reflux for 1 h. The mixture was cooled to rt and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x). The combined organic extracts were dried (MgSO<sub>4</sub>), concentrated under reduced pressure, and purified via SiO<sub>2</sub> flash column chromatography (1:1 EtOAc/hexanes with 1% TEA) to yield 0.333 g (80%) of the title compound as a pale yellow oil: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.28 – 7.20 (m, 5H), 6.71 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.47 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.39 (d, *J* = 2.1 Hz, 1H), 5.00 (s, 2H), 4.20 (m, 1H), 4.13 (t, *J* = 4.5 Hz, 1H), 3.82 (s, 3H), 2.76 (dt, *J* = 12.4, 2.9 Hz, 1H) 2.40 (dt, *J* = 10.1, 2.1 Hz, 1H), 2.38 – 2.30 (m, 4H), 2.23 – 2.01 (m, 3H), 1.81 (ddd, *J* = 10.4, 8.2, 5.2 Hz, 1H), 1.64 – 1.43 (m, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 149.70, 146.92, 141.99, 128.39, 127.49, 126.57, 121.20, 118.21, 111.70, 81.60, 64.5, 63.99, 56.16, 53.24, 51.32, 47.65, 46.24, 38.07, 32.68, 28.01, 24.65, 22.67, 14.12; IR (neat) 3563, 2932, 1515, 1279 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>23</sub>H<sub>30</sub>NO<sub>3</sub><sup>+</sup>: 368.2220, found 366.2224.



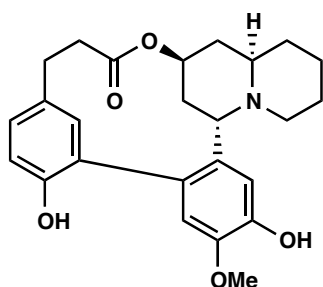
*3-(4-(Benzyloxy)phenyl)propanoic Acid (3.35).* Methyl 3-(4-(benzyloxy)phenyl)propanoate (11.9 g, 44.2 mmol, 1.00 equiv) was dissolved in MeOH (110 mL). KOH (0.4 N, 221 mL) was added and the reaction was allowed to stir until

completion (4 h). The reaction was diluted with H<sub>2</sub>O and acidified with 10% HCl until a pH of <1 and extracted with EtOAc (3x). The combined organic extracts were washed with brine, dried (MgSO<sub>4</sub>), and concentrated under reduced pressure to give 9.93 g (88%) of the title compound as white solid. The crude material was carried forward without purification. The spectral data was identical to that reported in the literature.<sup>361</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 11.33 (bs, 1H), 7.53 – 7.31 (m, 5H), 7.14 (d, *J* = 8.5 Hz, 2H), 6.93 (d, *J* = 8.6 Hz, 2H), 5.06 (s, 2H), 2.92 (t, *J* = 7.7 Hz, 2H), 2.67 (t, *J* = 7.7 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 173.78, 156.69, 137.23, 133.00, 129.33, 128.64, 128.00, 127.58, 114.98, 69.1, 35.5, 29.5.



(±)-4-(3-(Benzyloxy)-4-methoxyphenyl)octahydro-1H-quinolizin-2-yl-3-(4-(benzyloxy)phenyl)propanoate (**3.36**). Carboxylic acid **3.35** (0.174 g, 0.680 mmol, 1.00 equiv), DMAP (0.025g, 0.20 mmol, 0.30 equiv), and DCC (0.154 g, 0.746 mmol, 1.10 equiv) were dissolved into CH<sub>2</sub>Cl<sub>2</sub> and the resulting mixture was stirred at rt for 15 min. A solution of quinolizidinol **3.34** (0.250 g, 0.680 mmol, 1.00 equiv) in THF was added dropwise and the resulting solution was allowed to stir for 24 h. The resulting mixture

was poured into brine. The organic layer was separated (CH<sub>2</sub>Cl<sub>2</sub>), dried, concentrated under reduced pressure, and purified via SiO<sub>2</sub> flash column chromatography to yield 0.346 g (84%) of the title compound as a yellow oil: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.51-7.32 (m, 10H), 7.14 (d, *J* = 8.5 Hz, 2H), 6.96 (d, *J* = 8.6 Hz, 2H), 6.70 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.51 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.39 (d, *J* = 2.1 Hz, 1H) 5.34 (m, 1H), 5.08 (s, 2H), 5.06 (s, 2H), 3.85 (s, 3H), 2.94 (t, *J* = 7.7 Hz, 2H), 2.76 (dt, *J* = 12.4, 2.9 Hz, 1H), 2.62 (t, *J* = 7.7 Hz, 2H), 2.42 (dt, *J* = 10.1, 2.1 Hz, 1H), 2.39 – 2.28 (m, 4H), 2.23 – 1.95 (m, 3H), 1.79 (ddd, *J* = 10.4, 8.2, 5.2 Hz, 1H), 1.64 – 1.43 (m, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 173.99, 157.51, 149.61, 147.02, 141.89, 137.33, 133.00, 129.24, 128.74, 128.31, 128.03, 127.58, 127.50, 126.57, 121.26, 118.22, 114.99, 111.70, 81.60, 69.10, 64.53, 63.99, 56.11, 53.29, 51.30, 47.52, 46.41, 38.51, 35.91, 33.02, 30.02, 27.65, 24.32, 22.69, 14.31; IR (neat) 2944, 1741, 1495, 1251 cm<sup>-1</sup>; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>39</sub>H<sub>44</sub>NO<sub>5</sub><sup>+</sup>: 606.3214, found 606.3210.



**dihydrolyfoline 3.1**

(±)-*Dihydrolyfoline* (**3.1**). See **2.145a** and **2.125** for procedures. Seco-dihydrolyfoline **3.36** (0.042 g, 0.10 mmol) yielded 0.004 g (10%) of the title compound as a pale yellow

solid. The spectral data is consistent with that reported in the literature.<sup>362</sup> <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 6.94 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.87 (s, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.79 (s, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 4.88 (br s, 1H), 4.00 (d, *J* = 11.2 Hz, 1H), 3.76 (s, 3H), 2.99 (td, *J* = 13.4, 2.6 Hz, 1H), 2.94 (dd, *J* = 13.5, 2.6 Hz, 1H), 2.80 (d, *J* = 12.5 Hz, 1H), 2.70 (ddd, *J* = 13.5, 5.3, 2.8 Hz, 1H), 2.53 (ddd, *J* = 12.5, 5.3, 2.8 Hz, 1H), 2.34 (d, *J* = 15.0 Hz, 1H), 2.29 (td, *J* = 10.2, 4.0 Hz, 1H), 2.18 (td, *J* = 12.8, 2.5 Hz, 1H), 1.99 (ddd, *J* = 15.0, 6.5, 3.2 Hz, 1H), 1.65 (br t, *J* = 13.5 Hz, 2H), 1.56 (br d, *J* = 15.0 Hz, 1H), 1.44 (d, *J* = 12.1 Hz, 1H), 1.16-1.10 (m, 1H), 0.98-0.75 (m, 3H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 174.81, 152.32, 147.00, 146.46, 132.83, 132.02, 131.61, 129.05, 127.53, 126.01, 116.40, 114.04, 112.63, 71.07, 57.29, 55.91, 50.12, 47.88, 38.91, 38.80, 34.47, 32.50, 26.16, 25.74, 19.11; IR (neat) 2973, 1749, 1491, 1231; HRMS (ESI+) *m/e* calc'd for [M+H]<sup>+</sup> C<sub>25</sub>H<sub>30</sub>NO<sub>5</sub><sup>+</sup>: 424.2118, found 424.2106.

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