Syntheses and Biological Studies of Marine Terpenoids Derived from Inorganic Cyanide

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[†]

Abstract

Isocyanoterpenes (ICTs) are marine natural products biosynthesized through an unusual pathway that adorns terpene scaffolds with nitrogenous functionality derived from cyanide. The appendage of nitrogen functional groups–isonitriles in particular–onto stereochemically-rich carbocyclic ring systems provides enigmatic, bioactive molecules that have required innovative chemical syntheses. This review discusses the challenges inherent to the synthesis of this diverse family and details the development of the field. We also present recent progress in isolation and discuss key aspects of the remarkable biological activity of these compounds.

1 Introduction

A variety of nitrogenous terpenes have been isolated from marine organisms–sponges and tunicates–that retain vestiges of a biogenesis involving inorganic cyanide. These compounds are defined by terpenoid carbon scaffolds decorated with functionality revealing their unusual biosynthesis. The isonitrile, formamide, isocyanate, or isothiocyanate functional groups embedded in these terpenes impart biological and chemical properties distinct from more common oxygenated terpenes. This class of molecule is significant and growing (>130 structures), and has been a source of innovation and discovery in chemistry and biology for over 3 decades.

Various aspects of the chemistry and biology of these natural products has been reviewed previously.[i,ii,iii,iv] In particular, a 2004 review by Garson in this journal provided extensive discussion, particularly of biosynthetic considerations.[v] The present review will provide an update of the field and provide expanded discussion in two areas: (1) an overview of their pharmacology with particular focus on recent biological disclosures and antimalarial activity; and (2) the chemical synthesis of complex isonitrile-containing or -derived natural products. Syntheses will be analyzed according to C-C framework construction and installation of the key C-N bonds–a didactic exercise meant to illustrate advances in synthesis and methods over the last 4 decades. The overall goal of this review is to provide an overview of modern aspects of the synthesis of these molecules and their potential biomedical applications, as well as to point out unaddressed questions for the future.

Shown in Figure 1A are representative members of the key structural classes. The unusual biosynthetic nitrogen incorporation step is generally thought to involve an *N*-selective hydroisocyanation of unsaturated terpenes (ionization and capture of the corresponding

alcohol is also a possibility).[vi] The nitrogen source can be either cyanide or thiocyanate ions, though these inorganic species can interconvert through enzymatic processes (Figure 1B).[vii] The conversion of the initially formed isonitriles and isothiocyanates to formamides, amines, and isocyanates has not been studied in great detail and may arise, at least in part, through non-enzymatic reactions. Because the nitrogen functional groups of ICTs are not generally involved in gross, skeleton-forming biosynthetic steps, ICTs belong more within the terpene family of metabolite than with the alkaloids, even though the presence of basic amine groups within some of these molecules could place them in the latter class.

The functional groups contained within these molecules immediately raise questions regarding their reactivity in the biological milieu. Isonitriles are of particular interest due to their extensive associated biological activity (discussed in detail below). As shown with key examples in Figure 1C, isonitriles undergo a range of chemical transformations. These reactions find broad utility in a wide array of synthetic and biomedical applications, though our understanding of their role in the context of these natural products is still in its infancy. In addition to extensively studied reactions, such as protonation, Ugi and Passerini reactions, and metal coordination, recent reports have employed less well-known isonitrile reactivity for various applications. These efforts include the demonstration that isonitriles undergo a [4+1] cycloaddition with tetrazines in water with applications as a potentially bioorthogonal "click" bioconjugation approach.[viii] Isonitriles can polymerize to form helical structures in the presence of certain transition metals (e.g. Ni(II) and were recently shown to form hydrogel structures that mimic the mechanical response of key cytoskeletal proteins.[ix,x] Finally, Danishefsky and coworkers recently showed that isonitriles and carboxylic acids react to form *N*-formyl amides, with application for the synthesis of complex peptidic structures.[xi]

2. Recent Progress in Isolation

Since the most recent review, a number of new structures have been disclosed expanding the landscape of structural diversity of ICT and ICT-derived natural products. We particularly highlight studies where the reactivity patterns of the characteristic functional groups (i.e. isonitrile, isocyanate) are intimately involved in isolation and structural characterization efforts.

A number of bisabolene sesquiterpenes have been isolated (Figure 2). An isolation effort from "twilight sponges", isolated at depths below 50 m, produced a number of active extracts and resulting active compounds validating this unusual source material.[xii] These studies identified a new bisabolene derivative theonellin isocyanate **13**, as well the known isonitrile and isothiocyanate congeners. Other efforts obtained a variety of compounds in which the acyclic portion of the bisabolene scaffold is extensively oxidatively modified. These efforts include the isolation of epoxides, **14** and **15**, as well as a large number oxidatively modified structures in a single isolation study from *Axinyssa* sponges, termed the axinyssines A-L, **16** to **27**.[xiii,xiv] Relative and absolute assignments were obtained through mix of derivatization, NMR, MS, and CD experiments. In several instances the stereochemical assignment at C7, the position adjacent to the cyclohexane ring, remains to be defined.

A number of isolation efforts have identified additional sesquiterpene ICTs (Figure 3). The unusual cyclopentenone-containing structure, 3-oxo-axisonitrile **29**, was isolated from an *Acanthella* sponge.[xv] Zubía and coworkers disclosed a significant series of new bicyclic sesquiterpenes, the axinisothiocyanates, **31** to **44**, from sponges of the *Axinyssa* genus.[xvi,xvii] Among these is the nitrile-containing compound axinynitrile (**30**), the structure of which was verified by semi-synthesis. Notably, these highly substituted decalins are extensively oxygenated, including with peroxides, which is unusual among ICTs. Several other isolation efforts have provided isothiocyanates, formamides, and isocyanates, i.e. **45** to **48**, that complete the standard functional group tetrad (isonitrile, isothiocyanate, isocyanate and formamide) common with these compounds.[xviii, xix, xx] One of these efforts appeared to isolate new diastereomers within the cadinene group of sesquiterpenes,[xx] however subsequent synthetic efforts failed to validate the proposed structure and further efforts appear to be required.[xxi] Also quite significant is a systematic survey that compared the different constituent sesquiterpenes from various *Acanthella cavernosa* populations, which were taken from different isolation sites.[xxii] These studies included critical efforts to clarify uncertainties in the absolute and relative structures of several previously proposed structures. Consequently, some caution should be taken in evaluating the isolation literature for these isocyanoterpenes, since assignment of stereochemistry in particular is challenged by overlapping signals in associated spectra.

The diversity of diterpenes has also significantly expanded in recent years. The cavernene natural products, **49–52**, as well as additions to the kalihinol and kalihinene families, **53–58**, were reported through isolation efforts from *Acanthella cavernosa* (Figure 4A).[xxiii,xxiv,xxv] Notably, kalihinols M-T (**59–66**) were obtained from the South China Sea in a bioassay guided fraction effort targeted at biofouling. These compounds show a range of anti-biofouling activity, including several compounds in the submicromolar range.[xxvi] The variety of amphilectane structures has also grown, as shown in Figure 4B.[xx,xxvii,xxviii,xxix,xxx] Of particular interest are the methylamine and formamide, **77–78**, variants of the isoneoamphilectane scaffold, which were obtained from a *Svenzea flava* sponge and shown to display moderate antitubercular activity (MICs between 6 and 32 µg/mL against a *M. tuberculosis* $H_{37}RV$).[xxxi] Also quite notable was a significant reassignment effort of the absolute and relative stereochemistry of several previously assigned[xxxii] compounds through a combination of X-ray and Mosher ester analysis.[xxxiii]

Recent findings point out the role isonitrile reactivity can play in the biosynthesis, and isolation, of these molecules. Rodriguez and coworkers reported the isolation, structure, activity, and semisynthesis of the unusual β-lactam substituted amphilectene, monamphilectine A (**84**, Figure 5a). The natural product **84** was prepared in a–presumably biosynthetic–single step from amphilectene **8** through Ugi reaction with formaldehyde and β-alanine. The anti-malarial activity of β-lactam **84** is somewhat diminished relative to **8** (0.60 vs 0.04 µM against *Plasmodium falciparum* strain W2).[xxxiv] Nevertheless, given the central role of β-lactam antibiotics in medicine, it quite possible these new structures will ultimately be found to have useful activity.

A recent result reveals the care required in the isolation of these potentially reactive natural products. Compound **86** was obtained from an *Axinyssa* sponge and ultimately shown to be

an artifact of the isolation process (Figure 5b). This compound results from a Passerini reaction occurring during isolation between known sesquiterpene 3-isocyanotheonellin, **85**, acetone, and long-chain alkyl carboxylic acids.[xxxv]

Garson and coworkers suggested an intriguing biosynthetic hypothesis regarding the origin of a significant, and growing, group of urea-linked sesquiterpenes.[xxxvi,xxxvii,xxxviii] These ureas, exemplified in Figure 5c with halichonadin A, **89**, are isolated from marine sponges of the genus *Halichondria*. It is suggested that these arise through non-enzymatic addition of amines, formed from isonitriles through hydration and decarboxylation, to isocyanates. This suggestion is based on the isolation of an isocyanate and the isonitrile congener from the same natural source. Model studies verified the general underlying reactivity. These results suggest that halichonadin natural products are derived from ICTs and provide an example of non-enzymatic reactivity in biosynthesis.

Metal chelation represents another form of isonitrile reactivity that could conceivable play a key role in the biology of these compounds. In this context, a quite unusual copper(I) complex of halichonadin C (**91**) was isolated from the *Halichondria* sponge (Figure 5d). The authors report the chemical interconversion of the demetalated parent natural product halichonadin C (**90**) and its copper complex.[xxxix]

3. Chemical Synthesis of Isocyanoterpenes

3.1 Introduction

Retrosynthetic analysis of a molecule is guided by higher-level strategies that dictate the identity and order of transforms, intermediate targets en route, and occasionally the starting materials of the synthesis.[xl] For example, functional groups and their relative positions on the carbon skeleton of a molecular target provide useful 'handles' to guide the target's initial dissection.[xli] If the target is a naturally-occurring molecule, then biosynthesis–a known pathway,[xlii] or a reasonable, hypothesized pathway–can inform the choices made in retrosynthesis. Within the alkaloid classes of natural products, biosynthesis-guided retrosynthesis is an established higher-level strategy that is easily recognized and employed, since the overwhelming majority of C-N bonds in these molecules are synthesized by imine/iminium chemistry, and this chemistry is historically simple to execute in the laboratory.[xliii] Therefore the occurrence of isocyanoterpenes (ICTs)–nitrogenous, marine terpenoids whose C-N bonds are apparently derived from carbocations,[xliv] not imines–present a significant challenge to synthesis.

Apart from the challenges imposed by their aberrant biosynthesis, the problems encountered in ICT chemical synthesis diverge between the major subclasses. For instance, among the amphilectenes and adocianes (or cycloamphilectenes, **7–9**, Figure 1A-II) the absence of heteroatomic functional groups aside from widely spaced isonitriles limit the number of easily-identified polar disconnections.[xli] In contrast, the dense functional groups within the kalihinols (**10–12**, Figure 1A-III) provide a range of viable retrosynthetic transforms but chemoselectivity then limits the availability of corresponding chemical reactions. Chemical synthesis of any subclass must address the challenge of installing the isonitrile functional group.

Almost without exception, the isonitrile function is installed at the end of the synthesis. Therefore, the tactics used for isonitrile installation determine significant components of the global strategy since downstream maneuvers are influenced by the isonitrile precursor functional groups. Inasmuch as these functional groups are the sole heteroatoms in the target, their efficient utilization without introduction of superfluous functionality (functional group addition, FGA) is crucial. Therefore, before discussing any specific syntheses, the general approaches to isonitrile installation will be summarized.

3.2 C-N bond formation

Most naturally occurring, marine ICTs contain stereogenic *tert*-alkyl isonitriles and their derivatives (amines, amides, isocyanates, isothiocyanates), although some contain *sec*-alkyl substitution. The sec-alkyl amino groups are generally and easily established on the carbon scaffold by reductive amination of the corresponding ketone (Figure 6a),[xlv,xlvi] or S_N2 displacement of a secondary leaving group with a nitrogenous nucleophile.[xlvii] However, introduction of stereogenic *tert*-alkyl isonitriles, amines, and amides is challenging and has required diverse strategies to achieve its efficient and stereoselective introduction (Figure 6b). The Curtius rearrangement has been utilized by Piers,[xlviii,xlix,l] Miyaoka/Yamada,[li] Asaoka,[lii] and Mander[liii] to introduce the C-N bond with the correct stereochemistry based on the high stereospecificity associated with this reaction. The stereochemistry of the product isocyanate is specified by the stereochemistry of the precursor carboxylic acid, which is easy to establish by stereoselective alkylation (see discussion below). However, this strategy works best when the carboxylate is retained throughout the synthesis; otherwise its installation from the corresponding dehomologated ketone consumes several steps. An alternative approach devised by Wood establishes the correct stereochemistry via reductive cleavage of an aziridine.[liv]

Variations on the Ritter reaction also quickly establish the tertiary stereogenic isonitriles by capture of carbocations derived either from alkenes or tertiary alcohols and their derivatives. Most reports utilize the attack of a solvent separated ion pair,[lv,lvi,lvii] which means that existing, proximal stereocenters are necessary to induce any stereoselectivity. There is a single and remarkable report by Albizati[lviii] where the stereochemistry of the C-N bond is determined not by an ion pair, but by attack on a nascent carbocation (or by displacement of a C-Hg bond, *vide infra*). Finally, a recent report from Shenvi and coworkers describes a stereospecific tertiary alcohol displacement (via an intermediate perfluoroester) to form isonitriles.[lix]

3.3 Substituted or embedded decalin motif

Treatments of conformational analysis by organic chemistry textbooks usually describe the lesser stability of *cis*- versus *trans*-decalin (**92c** vs. **92t**) due to more gauche butane interactions in the former (three) versus the latter (none).[lx,lxi] Occasionally, the corresponding ketone, 1-decalone (**93c** or **93t**), is discussed also; this molecule prefers overwhelmingly the *trans*-ring fusion upon epimerization of the α-carbon.[lxii] Therefore, the *trans*-decalin motifs embedded in the largest families of ICTs (amphilectenes/adocianes, kalihinols, miscellaneous sesquiterpenes) would appear upon cursory analysis to be simple, and equilibration to favor the *trans*-ring junction by wide margin to be a foregone

conclusion. However, even slight variations from 1-decalone perturb the equilibrium to either inconvenient mixtures of stereoisomers (**94c**:**94t**, 1.4:1) or even towards a bias for *cis* (**95c**:**95t**, 4.6:1). Therefore, the planning phase for synthesis of polydecalin ICTs should include a careful conformational analysis of the thermodynamically preferred configuration at equilibrium. Some examples benefit from a preference for the targeted stereochemistry upon epimerization (**96**→**97**,[lvi] **98**→**99**,[lxiii] ratios are not given but high selectivity is implied in experimental details). Other cases suffer from the production of inconvenient mixtures (**100**→**101**,[lxiv] **102**→**103**;[liv] in the latter case, Wood solves this problem in a subsequent step, *vide infra*). In a final example, Vanderwal attempts to form the all-*trans* tricyclic core of the amphilectenes and adocianes via a transannular Michael reaction,[lxv] according to the report by Swaminathan that **105** is formed preferentially from **104**.[lxvi] Vanderwal determined that **105** was misassigned, and the Michael reaction instead produces **106** and **107**, which contain stable, mixed *cis*- and *trans*-decalin motifs, as proven by X-ray analysis. Furthermore, **106** and **107** are kinetic traps; their attempted equilibration does not lead to **105**.

The point is that the all *trans*-ring junctures should not be dismissed offhand as trivial, nor are epimerizable stereocenters clearable[xl] at every stage of the retrosynthetic analysis. Instead, careful planning should identify at what stage the decalins might be equilibrated to favor the desired stereochemistry.

3.4 Overview

The differing strategies for isonitrile installation position the molecule for dissection along numerous possible retrosynthetic branches, depending on the functional group used as an isonitrile precursor. Below, we compare the different ways in which subclasses of these molecules have been analyzed, and then discuss the syntheses as actually executed. In most cases, we take the analysis at face-value, even though a chemical synthesis almost never proceeds exactly as designed (also, the assumption is made that a retrosynthetic analysis was utilized in all cases, which may be an incorrect framework imposed by the authors).

The syntheses are organized roughly by subclass and within those groups presented mostly in chronological order. Understanding the chronology helps to properly assign credit to conceptual forerunners. In almost all cases, the targeted molecule is drawn initially in three dimensions to convey the actual geometry of the bonds and how this geometry keys certain retrosynthetic transforms (a symbol indicating rotation of Cartensian coordinates orients the reader, e.g. see Figure 12, **2** ≡ **2**). Only in a few cases are multiple synthetic steps replaced with a simple step count and yield; instead, each synthesis is covered thoroughly. Even though this level of detail yields large schemes, the larger format allows for the laboriousness of a sequence to be appreciated and also serves an educational role: learning many reactions (and how to avoid using so many!). Not every synthesis is covered; the remaining syntheses and relevant synthesis studies may be found in references lxvii–lxxxiii.

3.5 Miscellaneous sesquiterpenes

3.5.1 (–)-axisonitrile-3 (4)—Until recently, the first chemical synthesis of a marine isocyanoterpene cannot have been said to belong to Caine and Deutsch,[xlvii] since these

chemists produced the enantiomer of (+)-axisonitrile-3, one of the earliest isolated marine isocyanoterpenes. However, in 2011 Prawat and coworkers isolated (−)-axisonitrile-3 (**4**) from a marine sponge (*Halichondria* sp.),[lxxxiv] providing Caine and Deutsch the honor post-factum.

Although the targeted molecule contains a secondary isonitrile, its biosynthesis probably follows a variation of the Garson hypothesis (Figure 8),[xliv] since **4** can be imagined to arise from a cationic fragementation and nucleophilic capture of cyclopropane **108**. Indeed, De Rosa *et al.* have isolated (−)-cubebol (**109**) and axenol (**110**) from the marine alga *Taonia atomaria* and proposed their biosynthetic relationship via hydrolysis of **109**.[lxxxv] A related anionic fragmentation lies at the heart of Caine's synthesis of **4**.

The Caine strategy to access **4** is unusually beautiful, and relies, surprisingly, on steroid chemistry.[lxxxvi] Specifically, the synthesis derives some inspiration from the work of Piers (another contributor to isocyanoterpene chemistry, see Section 3.6.2) who demonstrated the stereoselective reductive cleavage of lumiketones related to lumicholestenone.[lxxxvii] The contributions of Caine and Deutsch to this area include expansion of these reductive fragmentations to vinyl cyclopropanes and application to the synthesis of **4**. Utilization of a vinyl cyclopropane rather than a cyclopropyl ketone is crucial, since the former leads directly to the correct trisubstituted alkene regioisomer (**111**), whereas derivatization from the corresponding carbonyl would require regiochemical control over enolization. The cyclopropane **112** would arise from the lumiketone rearrangement of **113**, which can be easily derived from dihydrocarvone (*see synthesis*). A final note: the authors install the isonitrile function via an S_N2 displacement of a sterically congested, secondary, neopentyl tosylate, which differs significantly from most strategies. Thirty years later, Kobayashi attempted to reproduce the reaction and failed, but discovered that a sodium cyanoborohydride reduction of the corresponding oxime established the same correct stereochemistry[lxxxviii] – details to keep in mind for future investigators.

The synthesis begins with the isolation of the tertiary alcohol intermediate in the Robinson annulation of dihydrocarvone (**114**) with methyl vinyl ketone, followed by isopropenyl hydrogenation with Adam's catalyst.[lxxxix] Treatment of **114** with catalytic sulfuric acid in acetic anhydride effects elimination of the hydroxyl and conversion of the nascent enone to the extended enol acetate. This intermediate is treated with *m*-CPBA under aqueous conditions to generate a labile epoxide that opens to form enone **116** with good (6:1) stereoselectivity. In order to access the cyclohexadienone substrate for rearrangement, the stereogenic alcohol is first protected as its methoxyisopropylidine ether (**117**). Unsaturation is carried out using selenation of the ketone enolate, followed by oxidative elimination to yield **113** (presumably the aqueous acidic workup[xc] to remove diisoproylamine after selenation also cleaves the methoxyisopropylidine ether; the authors do not comment). Irradiation of a dilute, room temperature dioxane solution of **113** with a low-pressure mercury lamp causes stereospecific rearrangement to **118**, equivalent to the classical photochemical rearrangement of santonin.[xci] Catalytic hydrogenation and Wittig olefination of **118** provide **112**, which is poised for the planned reductive fragmentation. Whereas carbonyls undergo single-electron reduction at cryogenic temperatures in liquid ammonia, alkene **112** requires more activation energy–a balmy 16 °C in ethylamine–to initiate

reduction. Although this energy barrier usually limits functional group compatibility,[xcii] there are no easily reducible groups in **112** and therefore the reaction proceeds cleanly to **111**. It is noteworthy that the stereogenic methyl of **111** possesses the thermodynamically preferred equatorial stereochemistry, even though Piers had observed selectivity for the equatorial-axial dimethyl cyclohexane **120** (see **119**→**120** + **121**).[lxxxvii] Both Caine's and Piers' systems yield inversion of stereochemistry, but the basis of this selectivity is unclear.

Regardless, **111** can be elaborated into the target structure first by tosylation to **122** and then S_N2 displacement with potassium azide in the presence of 18-crown-6, optimized conditions that minimized elimination. Lithium aluminum hydride reduction produced the corresponding amine **124**, which was converted to the isonitrile **4** using conditions developed by Corey[xciii] – the most commonly applied method for this conversion (*vide infra*).

3.5.2 (–) and (±)-9-isocyanopupukeanane (2) – Corey—The first chemical syntheses of this molecule were published back-to-back in 1979 by the groups of Corey[xlv] and Yamamoto[xlvi] (both manuscripts were received by the JACS editorial office by post on October 3rd from Honolulu, HI and Cambridge, MA, respectively – remarkable timing even considering they were submitted for simultaneous publication).

The biosynthesis of **2** is proposed[xciv,xcv] to traverse several carbocationic intermediates by a series of hydrogen- and carbon-shifts. While these reaction cascades are not unusual in terpene biosynthesis, they are still remarkable given the high energy of carbocations (*vide infra*) and therefore remain a vibrant research area today.[xcvi,xcvii] Formation of the isonitrile would proceed according to the Garson hypothesis[xliv] from the 9-pupukeanyl cation (**125**), which is hypothesized to derive from isomeric cation **126** via a hydrogen shift, in turn formed from twistane cation **127**. Cation **127** could be formed by Markovnikov cation-olefin cyclization from α-amorphenyl cation **128**, a commonly hypothesized cationic intermediate in the biosynthesis of diverse terpenes.[xcviii]

The evolving heuristics for retrosynthetic analysis being developed in the Corey laboratories at the time helped shape his strategy (originally racemic, *vide infra*) to access isocyanopupukeanane **2** (also evolving was the name for this approach for synthesis planning, which in this paper is called *antithetic* analysis, although *retrosynthetic* has since stuck).[xl] The first transform applied to **2**, conversion of the isonitrile to the ketone, is a common and almost universally conserved strategic move within ICTs (*vide infra*) for three important reasons. First, a stereocenter is removed, thereby reducing the complexity of the molecule. Second, a challenging functional group is removed, which simplifies the practical aspects of applying chemoselective transforms downstream. And third, the ketone allows many more transforms to be directly applied since this functional group's chemistry is so well explored. Corey's strategy takes advantage of one such transform: the alkylation of ketone **129** with a pendant electrophilic carbon, which cleaves a ring of maximal bridging by breaking an *exendo* bond, a strategic, high priority bond according to his retrosynthetic analysis guidelines (for detailed description of these rules and nomenclature beyond the scope of this review, see Ref. xl). This cleavage delivers a cis-fused 5–6 ring system **130** where the electrophilic methyltosylate is positioned endo and therefore proximal in space to

the ketone's alpha-carbons. The saturated ring system is retrosynthetically unsaturated and the stereogenic quarternary carbon of **130** is simplified to the ketone of indanone **131**. This broad stroke move is presumably based on the idea that the stereogenic isopropyl group would dominate other molecular features in controlling stereochemistry. Indanone **131** is derived from the arene **132**, which was synthesized in racemic form in 1979, but rendered scalemic (87% ee) in 2010.[xcix]

Preparation of enantioenriched starting material is accomplished by the development of a new chiral diene ligand by Brown and Corey for the rhodium-catalyzed enantioselective conjugate addition of aryl- and alkenyl boronic acids to enones (**133** + **132**→**135**).[xcix] Subjection of **135** to potassium hydroxide and bromine effects a haloform reaction to generate carboxylic acid **132**, which is converted to its corresponding acid chloride and cyclized via a Friedel-Crafts acylation to provide indanone **131**, thus intercepting the 1979 synthesis. The ketone can be homologated to **136** by the Van Leusen reaction with TosMIC;[c] a similar homologation is utilized 30 years later in a related class of ICT (see Section 3.7.1). Saponification and diazomethane esterification of **136** provides methyl ester **137** as an inconsequential mixture of diastereomers. The stereochemistry of the alpha carbon is set by methylation with good stereoselectivity (6:1) due to delivery of the methyl group to the ester enolate face opposite to the isopropyl group. Boron tribromide is used to remove the methyl protecting group of the phenol, and concurrently demethylates the ester. Reduction of indane **138** to the perhydroindane is accomplished with Nishimura's catalyst[ci] – a mixed oxide of rhodium and platinum prepared in analogy to Adam's catalyst, and based on the observations that colloidal rhodium black is effective to reduce aromatic rings.[cii] Although a pressure of 200 atmospheres of hydrogen is required, the major product **139** (37% yield, no d.r. given) possesses the correct cis-hydrindane ring fusion and its alcohol has cyclized into a lactone, likely a result of the catalytic perchloric acid used. Reduction of the lactone provides a diol that is chemoselectively tosylated at the least hindered primary alcohol, which allows the remaining secondary alcohol to be oxidized with pyridinium chlorochromate (PCC, developed[ciii] by Corey and Suggs in 1975), yielding ketone **130**.

When **130** is deprotonated with LDA in THF, the kinetic enolate reacts to form a four-membered ring, which is presumably formed slower than the six-membered ring, but closes faster than proton exchange between the enolate and the product. Therefore, treatment of **130** with strong base under equilibrating conditions (*t*-BuOK, *t*-BuOH) produces **129** as the major isomer. Installation of the isonitrile with the correct stereochemistry involves a four–step process of: hydroxylamine condensation to an oxime; reduction with Nishimura's catalyst[ci] to cleave the N–O bond and reduce the C=N bond; and finally *N*-formylation of amine **140** and formamide dehydration according to a procedure developed in the Corey labs[xciii] (and utilized in nearly all of the ICT syntheses) to yield 9-isocyanopupukeanane (**2**).

Important features of Corey's synthesis of **2** include the use of TosMIC to homologate a cyclic ketone, which finds use in subsequent ICT syntheses; the two step formylation/dehydration conversion of an amine to an isonitrile, which finds use in nearly every ICT synthesis; and the demonstrated ability of Corey's retrosynthetic analysis guidelines to provide workable and efficient (17 steps) laboratory syntheses. The unsolved problem of inducing asymmetry was met with a solution in 2010 by the use of a chiral diene ligand, thus

completing a thirty year cycle of problem identification, solution, and application – recursive logic that appears frequently in the Corey oeuvre.

3.5.3 (±)-9-isocyanopupukeanane (2) – Yamamoto—The synthesis of **2** by Yamamoto appearing in JACS one page after Corey's paper applies an entirely different strategy, although it intercepts the same ketone **129**, which is a logical precursor to the stereogenic isonitrile. The stereogenic isopropyl substituent derives from a vinyl isopropenyl group in **141**. The embedded [2.2.2]-bicycle keys an intramolecular Diels-Alder cycloaddition, which removes all consequential stereocenters but one (in **142**), an all-carbon quaternary center derived from a Claisen rearrangement of **143**.

The chemical synthesis begins with DIBAL reduction of enone **144** to yield a secondary allylic alcohol, which is heated in the presence of ethyl vinyl ether and mercuric acetate to produce aldehyde **145**. Addition of vinylmagnesium bromide to **145** produces an inconsequential mixture of 4 diastereomeric alcohols, which are protected as their tetrahydropyran ethers (yielding eight diastereomers!). Allylic oxidation of the cyclohexene is accomplished with chromium trioxide-pyridine complex, leading to eight enone diastereomers of **146**. Deprotonation and silylation leads to enolsilane **142**, the targeted diene for the intramolecular Diels-Alder cycloaddition, which occurs with heating in benzene in a sealed vessel at 160 °C. Subsequent treatment with aqueous acetic acid at elevated temperature removes both the trimethylsilyl and tetrahydropyran groups to produce tricycle **147**. After ketalization of the ketone with ethylene glycol and Corey-Kim oxidation[civ] of the secondary alcohol, all superfluous stereocenters are removed and the eight diastereomers of **146** converge to a single diastereomer **148**. The ketal is necessary to prevent subsequent addition of the isopropyl group to the other ketone. Thus, installation of the isopropyl group occurs over four steps: isopropenyl lithium addition to **148**, deketalization to yield **149**, alcohol elimination (to **150**), and diene hydrogenation with iridium black to provide tricycle **129**. Use of iridium led to a very high diastereomeric ratio (>98:2) favoring the correct geometry, whereas other catalysts led to lower stereoselectivity, likely a result of competitive alkene isomerization.[cv] Tricyclic ketone **129** is an intermediate in the Corey synthesis of **2**, and conversion to the target structure follows a similar path with slightly different conditions. Specifically, oxime formation is conducted in the absence of pyridine and therefore requires heating to produce **151**. Oxime reduction utilizes an iterative procedure of deoxygenation with low valent titanium, followed by imine reduction with DIBAL to amine **140**. Conversion of this amine into 9-isocyanopupukeanane (**2**) utilizes the same conditions as described in Caine's and Corey's syntheses above.

3.5.4 (–)-2-isocyano-trachyopsane (1) – Srikrishna—Reproducing the cationic reaction pathways of terpene biosynthesis is a major challenge in synthetic chemistry. Non-conjugated carbocations are extremely unstable and therefore impersistent (the lifetime of a tertiary carbocation in water has been estimated to be near the vibrational limit of 10^{13} sec^{-1}),[cvi] and almost always exist in equilibria with the corresponding alkene under synthetically relevant conditions.[cvii] Therefore, the idea by Srikrishna and coworkers to mimic two cationic biosynthetic steps in the synthesis of 2-isocyano-trachyopsane (**1**) was a bold proposition, albeit one founded on reasonable reactivity.

First, the isonitrile is excised through a cationic Ritter reaction, where stereochemistry is controlled by the concavity of tricycle **152**. The alkene equilibrium is inconsequential to the erasure of any adjacent stereocenters because they are bridgehead carbons. The bond network of **152** is established quickly through another cationic reaction, a Wagner-Meerwein shift, which simplifies the ring system by embedding a [2.2.2]-bicyclic motif (**153**). This new tricycle is dissected using a rhodium-catalyzed C-H insertion to establish a dissonant relationship between the two oxygen functional groups, whereas the consonant relationships can be easily established using tandem Michael reactions, ultimately arriving at carvone **154**. It should be noted that *ent*-**1** and formamide **159** (see chemical synthesis, Figure 14b) are both isolates of marine organisms, so a single synthetic route is bound to access the incorrect enantiomer of either **1** or **159**. Fortunately carvone is commercially available as either enantiomer, so the desired target is specified by the starting material with little extra expense.

The route to convert (−)-carvone (**154**) into the intermediate tricycle **157** (and (−)-9-pupukeanone)[cviii] had previously been published by Srikrishna[cix] and traverses a remarkably concise sequence. First, the carvone enolate attacks methyl methacrolate in a Michael fashion from the face away from the isopropenyl group, and the newly formed ester enolate engages the enone in a second Michael reaction to generate bicycle **155**. Saponification, acid chloride formation, and diazoketone installation proceed efficiently (82% overall) to yield **156**, which is treated with catalytic rhodium(II) acetate (catalyst loading not given) in refluxing dichloromethane to produce diketone **157** via C-H insertion. Borohydride reduction occurs selectively at the cyclopentanone on the convex face, followed by a palladium-catalyzed hydrogenation, which reduces the isopropenyl group to the stereogenic isopropyl found in **153** and **1**. When **153** is heated with camphor sulphonic acid in refluxing benzene, the transient carbocation undergoes a Wagner-Meerwein carbon shift to the more thermodynamically-stable tricycle **152**. A Ritter reaction with cyanotrimethylsilane (or trimethylsilyl cyanide, TMSCN) generates a formamide with stereochemical control of C-N bond formation caused by substrate bias (concave versus convex attack). Dithane formation is accomplished using ethanedithiol and iodine, which generates hydroiodic acid *in situ* and promotes nucleophilic attack and thionium formation. Finally, Raney nickel desulfurization generates (−)-2-(formylamino)trachyopsane **159**, which is dehydrated to (−)-2-isocyano-trachyopsane **1**, the enantiomer of the isolated material.

3.5.5 (±)-Isocyanotheonellin (85) – Ichikawa—The challenges and pitfalls of manipulating carbocations are evident in the synthesis of isocyanotheonellin (**85**) by Ichikawa.[cx] The target is dissected first by Julia olefination, and then by cationic C-N bond formation (Ritter reaction) to arrive at sulfone **160**. The order of these transforms is imperative. The Ritter reaction is difficult to achieve with site selectivity, and therefore the presence of multiple alkenes is problematic in this and related systems (see Ref. lix and cxi). Furthermore, unlike the S_N2 reaction, most S_N1 reactions of carbocations are not stereospecific and must rely on existing stereocenters in the substrate to direct stereochemistry. Therefore, if the architecture of a substrate leads to the incorrect stereoisomer, there is little recourse to correct it. This is the problem encountered by Ichikawa.

The synthesis begins with ketone **161**, the Diels-Alder adduct of methyl vinylketone and isoprene, which is converted to allyl alcohol **162** via Horner-Wadsworth-Emmons olefination (3:1 stereoselectivity) and reduction. The alcohol is transformed into the corresponding allylic bromide, enabling alkylation of sodium phenylsulfinate to generate sulfone **160**. At this point, the isobutilidine sidechain can be appended via Julia olefination, but a subsequent Ritter reaction is low yielding (38%, 63% based on recovered starting material) and more importantly the C-N bond formation favors the wrong diastereomer. The alternative Ritter addition to sulfone **160** offers a better yield (98%; fewer alkenes to protonate, electronic differentiation) and a marginal improvement in stereoselectivity, where now the two isomers **163a** and **163b** are produced in equimolar ratio. The isomers can be separated, and **163a** carried forward to the natural product **85**. Since, like most S_N1 processes, the stereoselectivity of the Ritter reactions depends on direction from the substrate, no better solution is available to Ichikawa.

3.5.6 (±)-α-bisabolyl amine (164) - Ichikawa—Ichikawa encountered a similar problem in his synthesis of α-bisabolyl amine (**164**),[cxii] an isolated hydrolysis product of the corresponding isonitrile, which is also a metabolite (**6**, Figure 1A-I). It had previously been shown that cyclization of nerolidol (**165**) with trifluoroacetic acid (TFA) or other acids promotes a cationic cyclization to multiple bisabolene isomers (e.g. **166**).[cxiii,cxiv] Similarly, if the cyclization is run in the presence of acetonitrile, bisabolyl cation (**167**) is trapped in a Ritter reaction, and bisabolyl amide **168** results, albeit in lamentable yield and as a 3:2 mixture of diastereomers. A three step hydrolysis procedure is known to convert the minor amide diastereomer into its corresponding α-bisabolyl amine (**164**).

3.5.7 Mercury-mediated amidation – Albizati—A fascinating example of a stereocontrolled Ritter reaction was reported by Albizati in his approach to isocyanoterpenes.[lviii] Subjection of **169** to a carbophilic mercury salt, mercuric nitrate $(Hg(NO_3)_2)$ initiates a polyene cyclization and capture of the nascent cation with acetonitrile solvent to provide intermediate nitrilium **170**. Not only does C-N bond formation occur with high stereoselectivity, but the correct equatorial stereochemistry of amides **171** and **172** is preferred, even though nitrile attack on solvent separated decalin carbocations usually provides the axial C-N bond[lix,cxv,lvii] (see Section 3.6.4). This preferred trajectory could be explained either by a delocalized carbocation or by a bridging mercury atom – either way its explanation is not trivial, and confounded by the finding that phenylselenium ions provide the axial amide corresponding to **171**.

3.5.8 (−)-α-bisabolyl amine - Shenvi—An alternative approach to achieve stereoselective capture of carbocations by nitriles is to start with chirality at the reactive center. Although it is generally true that substitution reactions that proceed via the intermediacy of a carbocation do not exhibit stereospecificity, there are isolated reports of stereospecific capture by attack at contact ion pairs.[cxvi,cxvii,cxviii] On the basis of this literature, the Shenvi laboratory developed a Lewis-acid catalyzed solvolysis reaction that is capable of inverting the stereochemistry of tertiary alcohols (as their ester derivatives) probably through attack of the contact ion pair.[lix] Using this reaction, the commercially available essential oil component bisabolol (**173**) can be converted to isocyanobisabolene

ent-**6** and α-bisabolylamine (−)-**164** with good diastereoselectivity and in excellent yield in just two and three steps, respectively. It is noteworthy that the trifluoroacetyl ester **174** is crucial for high stereoselectivity, but the basis for its superiority to other esters (acetyl, formyl) has not been determined.

3.5.9 (+)-α-bisabolyl amine - Ellman—A more generalizable solution to chiral *tert*-alkyl amines like **164** was published by Ellman, who uses his versatile chiral auxiliary to address the problem of stereocontrol at the *tert*-alkyl amine.[cxix] Instead of excising the C-N bond, the authors choose to remove the homoprenyl side chain to reveal sulfinimide **175**, whose cyclohexene can be dissected into imide **176**, which is the product of stereocontrolled alkylation of **177**.

Amidine **177** can be generated in good yield over 4 steps from orthoester **178** by first reaction with the Ellman auxiliary to provide imidate **179**, whose corresponding iodide will couple with the reagent derived from isopropenylmagnesium bromide and cuprous iodide to give **180**. Conversion of imidate **180** to amidine **177** is necessary for subsequent alkylation, since the electron withdrawing sulfinyl group renders the metalloenamide derived from **180** unreactive towards alkyl halides. Thus treatment of imidate **180** with morpholine in the presence of catalytic cyanide provides amidine **177**, whose corresponding metalloenamide is efficiently alkylated by allyl bromide with perfect diastereocontrol; addition of methylcerium chloride yields sulfinimide **176**. Ring closing metathesis using Grubbs' second generation catalyst produces cyclohexene **175**, and addition of homoprenyl lithium provides sulfinamide **181**, again with perfect stereocontrol for the correct diastereomer. Removal of the sulfinyl group is accomplished with hydrochloric acid and basification of the reaction delivers (+)-**164**. These isocyano-sesquiterpenes (**1,2,4,85,164**), although structurally disparate, possess similar challenges, mainly related to the problems of the nitrogen substitution patterns and stereochemistry imparted by their unusual biosynthesis. In contrast, the following sections analyze structurally similar isocyanoterpenes, which possess (by definition) similar challenges, but which are solved in very different ways, for better or worse.

3.6. Amphilectenes and adocianes

The amphilectenes and adocianes, also called cycloamphilectenes (Figure 20), are diterpenes (C20) composed of fused tri- and tetracyclic skeletons with isonitrile substitution conserved at C7 (occasionally at C8) and/or C20. There is minor variation of stereochemistry, alkene position, and methyl groups within this family, but generally its members are readily identifiable by their fused ring systems. The skeletons are stereochemically dense and usually consist of all-*trans* decalin motifs, though not exclusively. However, the all-*trans* stereochemistry is an unusual challenge since it limits the number of bonds susceptible to application of the Diels-Alder transform, which, when applied to cyclohexenes, gives rise to *cis*-stereochemistry, and therefore one newly formed center must be epimerized (see Figure 21 below). Furthermore, the *trans*-decalins are not always thermodynamically favored versus *cis*-decalins (see Section 3.3)–this configurational preference is surprisingly sensitive to substitution patterns and attempted equilibrations of *cis*- to *trans*-decalins are often met with poor diastereoselectivity.

3.6.1 (+)-7,20-Diisocyanoadociane (7) – Corey—Among the highest-priority transforms for 6-membered ring formation is the Diels-Alder reaction due to its potential to simplify complex bond networks to unsaturated building blocks.[cxx] Corey and Magriotis recorded the first synthesis of an adociane in 1987 and rely on sequential Diels-Alder reactions to assemble its network of repeating all-*trans* decalins, a challenge for retrosynthetic application of the selectively *syn*-facial Diels-Alder cycloaddition.[lvi] The first retrosynthetic 'move' is to excise the isonitriles: a daring S_N1 substitution transform is applied to arrive at the bis-tertiary alcohol **184**, which can be simplified to ketone **185**. The intermediacy of ketone **185** is crucial, since the stereocenters highlighted in blue can be ignored as they represent the thermodynamically preferred configurations accessible through epimerization. Thus ketone **185** can be further transformed via multiple functional group interconversions to **186**, in which a double bond has been installed to generate a retron for the Diels-Alder transform. Although the cycloaddition would be *syn*-facial, one of these stereocenters is later epimerized to the correct embedded *trans*-decalin. Since dienophile **187** itself contains a retron for the Diels-Alder reaction, an additional application of this transform to **188** (after some FGIs) reaches a relatively simple branched polyene **189**, whose synthesis is examined below.

The synthesis begins by appending a chiral auxiliary, (−)-menthol **190**, onto what will become the first section of the carbon scaffold, glutaric anhydride, to give a carboxylic acid that is transformed into its acid chloride and Stille coupled to vinyltributyl stannane to provide enone **191**. Standard ketalization of **191** is not possible, so a two-step workaround was devised whereby a combination of TMS phenyl selenide, iodine and ethylene glycol generate a β-phenylseleno ketal, whose selenide can be oxidized and eliminated to generated unsaturated ketal **192**. Michael addition of the enolate derived from **192** into methyl crotonate provides **193** in good yield and acceptable diastereoselectivity (8:1 threo:erythro and 4:1 selectivity relative to (−)-menthol). Selective reduction of the less sterically-shielded methyl ester is achieved with RedAl (sodium bis(2-methoxyethoxy)aluminumhydride) and the resultant primary alcohol is protected as its silylether. The menthol auxiliary is removed by LAH reduction, the primary alcohol is oxidized with PDC, and the resulting aldehyde is subjected to Vedejs-Wittig olefination[cxxi] to yield the *trans*-butadiene **189**. The Diels-Alder cycloaddition of ketal **189** occurs at 150 °C to provide the *trans*-decalin **188** selectively, whereas the Diels-Alder reaction of the corresponding ketone proceeds at room temperature[cxxii] but produces the *cis*-decalin, which cannot be quantitatively epimerized to *trans*- in related systems.[cxxii]

Conversion of the primary silyl ether to the requisite diene **195** was accomplished by fluoride-mediated deprotection, another PDC oxidation, and *E,E*-selective Horner-Wadsworth-Emmons olefination with phosphonate anion **196**. It was not reported whether the inverse-demand Diels-Alder cycloaddition of **195** was attempted, although an oblique reference to this possibility was made in the paper (changing the terminal substituent affects the stereoselectivity of the cycloaddition). Instead, DIBAL reduction of ester **195** and alkylation with benzyl bromide provides a terminal benzyl ether, which can be heated in toluene to effect the required cycloaddition, although the diastereoselectivity to produce **186** is modest because addition to the opposing cyclohexene face is competitive (1.5:1 d.r.).

The next several steps dehomologate **186** to ketone **96** via (1) benzyl ether hydrogenolysis, (2) alcohol oxidation to its aldehyde, (3) pyrrolidine enamine formation, and (4) oxidative cleavage of the enamine alkene with ruthenium tetroxide. At this point, the epimerizable locations adjacent to the western ketone can be used to advantage. Even though one cyclohexane ring is *cis*-fused, its equilibration to the *trans*-junction can be easily accomplished with sodium methoxide in methanol. Then, although methylation of the kinetic enolate preferentially forms the axial α-methyl ketone, an additional equilibration with sodium methoxide delivers the equatorial methyl. Finally, deketalization in the presence of acetone yields diketone **197**.

The endgame of the synthesis involves double methyl addition using methylcerium chloride and trifluoroacetylation in the presence of pyridine to establish the two tertiary centers. Since equatorial attack is highly favored in methylation, the bis-axial alcohol is produced almost exclusively. Ironically, however, this inherent stereoselectivity of the substrate causes two problems: (1) addition of methyl nucleophiles to the corresponding imines would generate the wrong stereochemistry at C7 for the amphilectene and kalihinol classes (this problem is encountered in nearly every subsequent synthesis), and (2) whereas the C7 alcohol must be inverted, the stereochemistry at the C20 alcohol must be retained. In audacious fashion, the problems are tackled head-on by ionizing the trifluoroacetyl esters **198** with titanium tetrachloride in the presence of excess TMSCN. This reaction leads to a mixture of four diastereomers in nearly statistical mixture and 70% total yield that are separated by HPLC. Over one-half of the mixture corresponds to equatorial/axial isonitriles, and assuming neither stereocenter influences the other, **7** would then constitute about 19% of the reaction mixture. So, whereas this synthesis provides a valuable answer to the question of absolute stereochemistry of **7**, further advances are needed to secure access to **7** efficiently.

Herewith, important features of this synthesis that significantly impacted future work: first, at a strategic level, the intermediacy of a branched polyene **189** has been chosen by multiple chemists working in the amphilectene/kalihinol family. Installation of this branch point prior to cycloaddition decisively places the burden of stereocontrol on the linear stereodiad of **189** and similar structures, and therefore the efficiency of the whole synthesis hangs on the accessibility of these branched diads. In later syntheses, we explore this access in more depth. Second, this synthesis is the first report of a Ritter-type reaction used in the synthesis of these marine isonitriles, with TMSCN in particular as the nitrogen source – an innovation that was further explored multiple times in the literature. The 42-step formal synthesis of **7** reported by Mander in 2006[liii] and the 32-step formal synthesis of **7** reported by Miyaoka in 2011[cxxiii] should be examined by the interested reader.

3.6.2 (±)-8,15-diisocyano-11(20)-amphilectene (8) – Piers—The second synthesis in the adociane/ amphilectene class was reported by Piers in 1989,[xlviii] only two years after Corey. However, the problems addressed in the synthesis of **8** are significantly different due to the absence of a fourth ring and the positioning of the isonitrile at an angular carbon. Piers removes the isonitriles from **8** using two Curtius reactions to arrive at the homologated diester **199**. This Curtius strategy is also utilized by Miyaoka and Yamada over a decade

later.[li] At this point, the carbon skeleton possesses three carbonyl functional groups, two of which are connected by a consonant pathway (blue numbering), and two of which are connected by a dissonant pathway (red numbering).[xli] Piers defies conventional wisdom and removes the middle carbonyl completely. Although this extinguishes the possibility of utilizing aldol or Michael disconnections, it also circumvents problems with chemoselectivity that might arise between multiple carbonyls. Further FGIs convert the northern cyclohexane partial retron into a Diels-Alder retron, cyclohexene **200**, which then allows dissection into diene **201** and acrolein. A Stille transform is then applied to arrive at vinyl triflate **202**, which simplifies to ketone **203** and halostannane **204**. The use of a vicinal difunctionalization to establish the stereodiad of **201** is an insightful strategy that rapidly builds **201** from the difunctional reagent **204** reported earlier by Piers,[cxxiv] and relies merely on the stereogenic methyl group to transmit all the stereochemistry of **8**.

The synthesis begins with carboxylation of 1-chloro-4-pentyne (**205**) to ynoate **206**, which is stereoselectively functionalized to stannane **207** via conjugate addition using stannyl-copper. Ester reduction, followed by alcohol protection and Finkelstein halide exchange delivers iodide **204**. Although alkylation of **204** by the enolate derived from organocopper addition to enone **203** is not efficient, the corresponding potassium enolate derived from deprotonation with KH cleanly generates the hindered quaternary center in **208**. Formation of diene **201** is accomplished in one pot by addition of palladium tetrakistriphenylphosphine into the crude reaction mixture containing the vinyltriflate **202** generated from deprotonation of **208** and reaction with *N*-phenyltriflimide. Refluxing **201** in the presence of excess acrolein effects a Diels-Alder reaction, and while the diastereoselectivity is poor (*ca*. 2:1 at C4) even after equilibration of the aldehyde stereochemistry, the correct isomer **200** can still be isolated in 58% yield. Sodium borohydride reduction of the aldehyde, tosylation of the alcohol, and deoxygenation via S_N2 displacement with Super-Hydride (lithium triethylborohydride) furnishes tricycle **209** in good overall yield. Allylic oxidation to install the conspicuously absent ketone is accomplished with the Corey-Fleet reagent,[cxxv] which was observed by Salmond and co-workers at UpJohn to significantly increase the rate of allylic oxidation in steroids.[cxxvi] Dissolving metal reduction of **210** delivers the thermodynamically-favored *trans*-ring fusion selectively, in accordance with observations by Sarrett[cxxvii] and Barton,[cxxviii] and methylenation with Lombardo's reagent[cxxix] furnishes **211**. Further FGIs–deprotection, Swern oxidation, epimerization to the equatorial aldehyde–transform the silylether into the corresponding aldehyde **212**, which is engaged in a Horner-Wadsworth-Emmons olefination to supply ester **213**. At this point, reduction of the unsaturated ester was unsuccessful (comments indicate poor selectivity for reducing this enoate versus the alkene or esters), so both esters are first demethylated with sodium benzeneselenoate. Dissolving metal reduction then chemoselectively reduces the unsaturated acid, and methylation of the trianion delivers diacid **214**. The double Curtius reaction produces an intermediate bis-isocyanoate, which is intercepted with 2-trimethylsilylethanol, presumably to aid in purification (the yield is only 52%). Removal of these newly formed Teoc protecting groups in **215** then provides the bis-amine which is converted into the bis-isonitrile **8** by a variation of the standard formylation/dehydration procedure, where a phosphorous(V) reagent replaces the commonly-used methanesulfonyl chloride.

Not only was this synthesis the first of the tricyclic amphilectane class, but it also laid the ground work for extension to a closely related member (8-isocyano-10,14-amphilectadiene) from intermediate **210**.[cxxx] Furthermore, Piers' work established the benchmark for efficiency in this class, which took 25 more years to improve upon.

3.6.3 (±)-7-isocyanoamphilecta-11(20),15-diene (183) – Miyaoka—Another

member closely related to **8** was synthesized by Miyaoka in 2011 using a retrosynthesis with similarities to the earlier Corey work. This similarity is especially evident in the latter part of the retrosynthesis and the choice to engage a branched diene in Diels-Alder cycloaddition. As now should be recognized as commonplace, the first move is to excise the isonitrile, in this case to ketone **216** (Miyaoka uses a procedure developed by Wood,[cxxxi,liv] which is covered in Section 3.7.2). The northern ring is cleaved in a stereoselective Michael reaction to **217**, and the decalin ring is dissected into **218** by a Diels-Alder transform similar to Corey's disconnection, where the branching stereodiad is installed early and controls the relative stereochemistry of the cycloaddition.

The branching stereocenters are derived from stereogenic α- and β-carbons adjacent to an ester (in this case a lactone). In Miyaoka's synthesis, these centers are established via *trans*-selective allylation of β-methyl velero lactone (**219**). Multiple FGIs are then applied to advance intermediate **220** to the requisite Diels-Alder precursor **218**. Of the 15 steps separating the two intermediates, 6 steps involve protecting group manipulation, and 4 cause oxidation state changes, leaving only 3 steps that build the carbon skeleton (2 other steps are also FGIs). The Diels-Alder reaction of **218** is accomplished upon silylation of the enone to provide **225** as the *cis*-decalin exclusively, in accordance with Taber's[cxxii] and Corey's observations (Corey used the corresponding ketal to alter the selectivity to favor the *trans*-decalin; it is likely that the presence of an alkene prevented thermodynamic equilibration of Corey's decalone;[cxxii] also see Section 3.7.1 below). Desilylation with tetrabutylammonium fluoride and epimerization using Hünig's base provides the *trans*-fused decalin diketone.

Removal of the trityl group is accomplished with hydrogenation over palladium hydroxide, and oxidation of the primary alcohol delivers aldehyde **226**. Homologation with a stabilized ylide provides an unsaturated ester that undergoes pyrrolidine catalyzed Michael addition to yield tricycle **227**. Wittig olefination occurs selectively at the southern ketone, and a Peterson olefination is required to install the western alkene. Fortuitously, attack also occurs on the ester and so methyl ketone **228** is also isolated along with carboxylic acid **229**, which can be converted in two steps to **228** via its Weinreb amide. From **228**, six additional steps are required for conversion into **183**. Miyaoka uses the Wood sequence[liv,cxxxi] (see Figure 31) for introduction of the equatorial isonitrile, and for those reactions, we must visit the kalihinols. First, however, a final synthesis from the amphilectene family.

3.6.4 (±)-7-Isocyano-11(20),14-epiamphilectadiene (9) – Shenvi—As the initial

report of a program to explore the chemistry and address the biological/mechanistic uncertainty of the isocyanoterpenes (discussed below), the Shenvi laboratory embarked on a synthesis of amphilectene **9**, which possesses the highest antimalarial potency of the tricyclic amphilectene class.[cxxxii] The initial design did not actually target **9** specifically, but rather aimed more broadly for a divergent synthesis, whereby multiple tri- and tetracyclic

amphilectenes might be accessed from a simple intermediate **230**–the synthesis of **9** would constitute a preliminary proof-of-principle. The isonitrile of **9** was transformed to the tertiary alcohol **231** on the basis of Corey's precedent, with the assumption that optimization of stereocontrol might be possible (the stereospecific S_N1 described in Section 3.5.8 had not been discovered at this point, and we had not realized that the presence of two alkenes would be problematic; see below). Excision of the methylene to ketone **232** establishes a 1,3-relationship between the isobutylene sidechain and the ketone such that a conjugate addition transform, along with methyl addition to the southern carbonyl, provide **230**. The most straightforward disassembly of **230** took advantage of sequential cyclohexene intermediates appearing via Diels-Alder transform, and simplified **230** and **231** to dienophile **234** and diene **235**. We felt this was not only a logical and concise analysis of **9**, but were also excited by the opportunity to explore methodological advances in isocyanation, and dendralene[cxxxiii] cycloaddition chemistry–an underutilized area in synthesis that can suffer from poor control of regio-and stereochemistry. Hybridization of Danishefsky's diene[cxxxiv,cxxxv] with the dendralene motif–the hybrid **235** was christened [3]-Danishefsky dendralene–was imagined to impart regiocontrol and consequently stereocontrol of iterative Diels-Alder reactions. At the outset, the identity of the activating group X was unknown (in fact, we naively assumed that a hydrogen atom would be competent, which it was not), but ultimately, a methyl ester was identified as the optimal functional group. The methyl ester not only prevented the intermediate cross-conjugated alkene from isomerizing into full conjugation, but it also activated the dienophile **234** for cycloaddition, whereas other groups necessitated heating, which caused competitive decomposition of dendralene **235**.

Synthesis of the dienophile **234** is accomplished in a short sequence from 3-buten-2-ol (**236**), which is heated with triethylorthoacetate in the presence of propionic acid to effect Claisen rearrangement and yield ester **237**. A subsequent Claisen condensation with methyl propionate yields β-ketoester **238**, which is unsaturated via selenation and oxidative elimination. It should be noted that **234** decomposes rapidly when stored neat, so this highly electrophilic dienophile is used directly without concentrating the reaction mixture. The [3]-Danishefsky dendralene **235** is synthesized in a three step sequence of iodination, Negishi coupling, and silylation; and like **234**, **235** is not stable over long periods of time. Therefore, a crude dichloromethane solution of **234** and **235** is concentrated *in vacuo*, which effects immediate cycloaddition to form **242** as an inconsequential mixture of diastereomers. Redissolution of **242** in toluene and treatment at 0 °C with catalytic ytterbium triflate causes elimination of *tert*-butyldimethylsilyl methyl ether to yield enone **233**. The second Diels-Alder is carried out at high temperature (likely a consequence of strain) in 1,2-dichlorobenzene to provide enone **243**. The strain inherent to **243** complicates removal of the methyl carboxylate since hydroxide will add conjugatively to the enone, so a modified Krapcho decarboxylation[cxxxvi] is used to demethylate and decarboxylate **243** to parent tricycle **230**–the *trans*-decalin is formed exclusively.

Conjugate addition is accomplished with isobutenylmagnesium bromide in the presence of copper bromide and trimethylchlorosilane, which protects the western ketone as its enolsilane, allowing selective addition of methylmagnesium bromide into the southern ketone to supply intermediate **232**, also as the *trans*-decalin. Notably, if conjugate addition

is effected prior to decarboxylation, both fused decalin motifs contain *cis*-ring junctions. The methylene is installed in a two-step interrupted Peterson olefination first to silylhydrin **244**; trifluoroacetylation is selective for the less hindered southern tertiary alcohol, providing **243**. Treatment of **245** with a solution of catalytic scandium triflate in cyanotrimethylsilane (TMSCN) inverts the stereochemistry of **245** at the tertiary ester to provide the equatorial isonitrile of **9** preferentially (though unknown at the time, this reaction is stereospecific and can invert single isolated stereocenters). Elimination of the silylhydrin is also accomplished under these conditions. Previously reported procedures for S_N1 isocyanation fail to convert **245** or **246a/b** (Figure 27) to **9**. For instance, the procedure of Tada[cxxxvii] and Kitano[cxxxviii] proceeds through a mixture of alkenes **247** (Figure 27) and Brønsted acid-mediated Ritter-type isocyanation does not differentiate between the electron-neutral olefins. The procedure of Corey[lvi] that was successful to generate stereoisomeric mixtures of 7,20-diisocyanoadociane (**7**, Section **3.6.1**) preferentially forms 7-*epi*-**9** and in low yield; studies on a model decalin and related S_N1 reactions suggest this stereochemical outcome derives from attack on a solvent separated ion pair, i.e. the substrates alone dictates the stereochemistry.[lix] This most recent synthesis of **9**, an admittedly simple member of the amphilectene class, achieves the shortest route yet to these bioactive compounds. The true value of the work lies in the invention of a demonstrably useful functionalized dendralene, and the initial disclosure of a stereospecific tertiary alcohol inversion reaction. The amphilectenes (and adocianes), while stereochemically complex, are functionally sparse and therefore chemoselectivity is not a significant problem. In contrast, the functionally dense kalihinols are represent the pinnacle of complexity within the isocyanoterpene class and a major challenge for chemical synthesis.

3.7. Kalihinols

There are two common names given to members of this class: the kalihinols and the kalihinenes, corresponding to a presence or absence of hydroxylation at C4 or C5 (see Figure 1A-III). The authors prefer the catch-all term 'kalihinol' for the entire family since the name (1) captures the unique C11-oxy-biflorin skeleton[cxxxix] common to and distinctive of the class, and (2) likely reflects the biosynthetic origins of the heterocyclic ring as derived from an alcohol. The functional group-density of the kalihinols and the close proximity of their numerous branching carbons have confounded attempts at an efficient synthesis. In particular, the sterically-encumbered heterocycle directly joined to a polysubstituted decalin motif poses a dilemma for its installation: how to situate this key bond joining the two systems with stereocontrol and efficiency. Ideally, vicinal stereocenters should be directly cleared to two achiral carbons,[xl] but this feat has never been achieved in the kalihinols. Either the rings are built around pre-existing stereochemistry (Miyaoka and Yamada), or one stereocenter controls the stereochemistry of its neighbor (Wood); these strategies are described in the forthcoming synopses.

3.7.1 (+)-Kalihinene X (11) – Miyaoka and Yamada—There have been only three syntheses of kalihinols, each molecule a slight variation on the family archetype. The first synthesis, reported by Miyaoka and Yamada,[li] is homologous to Corey's approach to **7**. One of the key challenges common to all the kalihinols is establishment of the C-C bond between the decalin motif and the *tert*-alkyl ether carbon within the heterocyclic ring

(tetrahydrofuran or -pyran, see **10** versus **11** or **12**, Figure 1A-III); this bond corresponds to the vicinal stereodiad link in the amphilectene/adociane class. Miyaokas's solution in this context is identical: generation of each ring separately from a linear motif containing pre-installed stereogenic centers. The retrosynthesis begins by simplifying the *tert*-alkyl formamide (the hydrolysis product of the corresonding isonitrile) to carboxylic acid **248** via a Curtius transform. The carboxylic acid is simplfied to ketone **249** via retrosynthetic homologation and the decalin dissected to triene **250** using the same Diels-Alder strategy as Corey. Functional group interconversion transforms applied to **250** produce tetrahydropyran **251**, which is simplified to stereogenic allylic chloride **252** by oxymercuration. The sequences 'build pyran' and 'build decalin' are highlighted since these tactics are applied in reversed order, and slightly more efficiently, in the subsequent Wood synthesis of kalihinol C[liv] (see below). Intermediate **252** is deconstructed to sulfone **253** and epoxide **254**, which is derived from geraniol by two enantioselective transforms to independently set the three chiral centers.

Geranyl acetate **255** is processed to diol **256** using a Kodama procedure[cxl] relying on enantioselective reduction of the intermediate hydroxy ketone corresponding to **256**. The same result can be obtained in one step by Sharpless dihydroxylation,[cxli] so the choice of the longer sequence is curious, but might have been due to financial constraints. Acetylation of the secondary alcohol of **256**, elimination of the residual tertiary alcohol and global deacetylation provide alcohol **257**. Selective silylation of the secondary allylic alcohol is not possible, so the primary alcohol is protected first as the TBS ether. Appendage of the TBDPS group onto the more sterically encumbered hydroxyl is followed by selective removal of the primary silyl ether to deliver alcohol **258**. Sharpless epoxidation using (−)-DIPT provides epoxide **254**, which upon alkylation by the anion derived from sulfone **253** yields **259** as a mixture of diastereomers. Desulfonylation with sodium amalgam and bisacetylation provide the polysubstituted 9-decenyl ether **260**. The stereogenic chloride is installed upon removal of the *tert*-butyldiphenylsilyl group with TBAF and S_N2 displacement using Appel conditions. In order to prepare the substrate for ring formation at the tertiary alcohol, the acetates are removed reductively with DIBAL, and the resultant primary alcohol is protected as a pivaloyl ester. At this point, 18 steps have been invested in the sequence to reach **252**, with no rings yielded as dividend.

The first ring is formed by oxymercuration to provide organomercurial **261**, and the carbon-mercury bond is reduced with sodium borohydride in the subsequence step to yield pyran **251**. This move circumvents the challenge of stereocontrolled heterocycle formation, since the cyclization does not form permanent stereocenters; all requisite stereochemical information is contained in linear precursor **252**. Elaboration of **251** to the intramolecular Diels-Alder substrate is conducted over 8 steps that contain two skeleton-building reactions. In accordance with Taber's[cxxii] and Corey's observations (Ref. lvi, footnote 11), the ketone derived from oxidation of alcohol **263** undergoes spontaneous cycloaddition at ambient temperature to provide primarily a *cis*-decalin, in this case **249**. Whereas most members of the kalihinol family contain a *trans*-decalin, the *cis*-decalin is represented in a handful of metabolites, including formamide **11**, which becomes the target of this synthesis and is now only 6 steps away. The ketone function in **249** again serves as a flexible synthon for the *tert*-

alkyl amines, and thus is homologated to nitriles **264** using TosMIC[c] (see also Section 3.5.2). The nitrile is alkylated on the convex face, corresponding to the correct stereochemistry for the targeted C-N bond. Notably, related studies suggest that analogous alkylation of the *trans*-decalin will not give the desired stereochemistry for the kalihinols.[cxlii] Reduction of the nitrile to the aldehyde and Pinnick oxidation to the carboxylic acid, followed by Curtius rearrangement and reduction of the resultant isocyanate with DIBAL produces the formamide, kalihinene X (**11**).

This first synthesis of a kalihinol exhibits several notable problems associated with the family. In concert with Wood's model study (see below) and Corey's synthesis of **7**, this work by Miyaoka and Yamada illustrates the challenge associated with installation of the equatorial C-N bond: multiple steps are required and its success relies on the concavity of the *cis*-ring fusion. Furthermore, the synthesis highlights the challenge of generating the stereopentad that delineates the contours of the directly- joined rings and fused rings of the kalihinol skeleton. Such challenges prolong the synthesis to a total of 35-steps.

3.7.2 (±)-kalihinol C (10) – Wood—A kalihinol model study was published by John Wood in 2001 followed by a synthesis of kalihinol C in 2004 (in addition to two excellent PhD theses by Ryan White[cxliii] and Gregg Keaney[cxliv]). His synthesis strategy overlaps substantially with Miyaoka's, although his model study was reported prior to the completed synthesis of **11** shown in the previous section. Wood also targets a more complex kalihinol that contains additional stereocenters embedded in the decalin, and a mixture of *sec-* and *tert*-alkyl isonitriles (see highlighted atoms in **10** and **265**). The strategy (see Figure 30) is also similar to Corey's in its use of an ester alkylation to generate the branching stereodiad (**270**) prior to a decalin-building Diels-Alder cycloaddition (**269→268**; one of these stereocenters is later erased). Two key differences to highlight between Wood's and Miyaoka's syntheses are: (1) the order of ring building (by building the decalin first, Wood circumvents some functional group incompatibility and therefore requires fewer protecting group manipulations); and (2) the use of stereochemical substrate control by Wood (this strategy obviates the tedious installation of stereocenters prior to ring building).

The synthesis of **10** commences with the alkylation of the dianion of **271** with alkyl bromide **272**, itself synthesized in three steps. This mixture of diastereomers (**273**) is carried through five steps of mostly functional group interconversion, including a Horner-Wadsworth-Emmons olefination to form the diene cycloaddition partner **274**. Deprotection of the TBS ether in **274** with fluorosilicic acid liberates the southern secondary alcohol for oxidation with PCC, initiating the Diels-Alder reaction at or below ambient temperatures to provide **275**. The nascent cyclohexene is epoxidized with dimethyldioxirane with high facial selectivity to generate epoxide **276**. Although the *cis*-ring junction mediates this high stereoselectivity, ultimately the *trans*-stereochemistry is preferred, since far more kalihinols possess this configuration. Therefore, **276** is partially epimerized with sodium methoxide to a 1.5:1 mixture with the *trans*-decalin epimer in excess, and this crude mixture is methylenated. Counterintuitively but conveniently, the *trans*-decalin **277** is produced almost exclusively from the epimerized mixture, whereas subjection of pure **276** to the same conditions provides 'a variable ratio' of *cis-* and *trans*-decalins.

One of the two stereocenters in the initially formed stereodiad is then erased to prepare for installation of the challenging *tert*-alcohol functionality. Dissolving metal reduction deprotects the benzyl ether and Dess-Martin periodane then oxidizes the secondary alcohol to the methyl ketone in **268**. The Wood group then developed a useful strategy to install the correctly disposed C-N bond: aziridation of alkene **268**, followed later in the sequence by reduction of the less-hindered C-N bond. It is noteworthy that the correct stereocontrol is achieved on this *trans*-decalin system, and telling that Miyaoka utilizes this sequence in a later synthesis of kalihinol A. Having established all the stereocenters in the decalin core, Wood now turns to the heterocyclic motif. Addition of the lithium acetylide derived from ethyl propiolate to methyl ketone **278** results in the anti-Felkin stereochemistry of the *tert*-alcohol with very high diastereoselectivity (98:2), and subsequent hydrogenation of the alkyne yields ester **279**. Monoreduction of the ethyl ester with DIBAL provides a lactol, which is olefinated with isopropylidine phosphonium ylide to yield alkene **267**. The tetrahydrofuran **280** is built using an oxyselenation that provides modest selectivity for the correct diastereomer (3:2). Elimination to **266** is effected upon *m*-CPBA oxidation.

At this point, both of the three-membered rings are opened to reveal the substitution patterns of the final target: the aziridine is reductively opened with Super-Hydride (LiEt₃BH), and the epoxide is opened at the less hindered position with sodium azide to provide intermediate **281**. Dissolving metal reduction then deprotects the tosyl amide and reduces the azide to the amine **265**. Double formylation/dehydration then establishes the bis-isonitrile of kalihinol C (**10**). Notable advances in this synthesis include a substantially more efficient entry into the polysusbtituted kalihinol framework than previously established, a multistep but useful strategy for installation of the equatorial isonitrile motif, and reconnaissance data regarding substrate stereocontrol, especially formation of the *O*-heterocycle.

3.7.3 (+)-kalihinol A – Miyaoka—Before concluding, Miyaoka's synthesis of (+)-kalihinol A (**12**), one of the most complex members of the kalihinol family, should be mentioned. However, the synthesis is so similar to that of kalihinene X by the same group that only the retrosynthesis will be analyzed here. In essence, the route uses the endgame of Wood's kalihinol C synthesis, and merges it with the kalihinene X entry. In this way, simplification of **12** to ketone **283** (which favors the correct *trans*-decalin over the incorrect *cis*-decalin by only a 3:2 margin upon epimerization) is accomplished using Wood's reconnaissance. The identical triene **250** as found in the synthesis of kalihinene X (**11**) is intercepted, and its synthesis relies again on pre-installion of all stereocenters on a linear chain **284**, which is synthesized in a nearly identical manner as chain **252** in Section 3.7.1. It is instructive to point out that the biosynthetic pathway to the diterpenoid kalihinols also begins with a linear precursor, but with no stereocenters preinstalled. Instead, the stereocenters of the carbon framework form during the cyclization of achiral tetraene **285**, geranylgeranyl pyrophosphate, once again highlighting the elegant reactivity that so eludes synthetic chemistry today.

Conclusion of synthesis section—Having surveyed most syntheses of the isocyanoterpenes, this section aims to convey the difficulties, idiosyncracies, benefits and

defects of current approaches. Clearly, an overriding theme in the field is the challenge of efficiency – both in synthetic steps and yield. For the kalihinols in particular, a unifying and divergent sequence that avoids the intermediacy of stereochemically complex, linear motifs would be ideal. The biosynthetically-aberrant isonitrile pharmacophore also remains a challenge. Although a biomimetic contact ion pair attack is advantageous in many cases, this approach may not be applicable to all substitution patterns, the isocyanohydrin motif of the kalihinols in particular. Furthermore, careful attention should be paid in the planning stage to points in the synthesis where the *trans*- or *cis*-decalin ring junctures might be obtained selectively through equilibrating epimerization. The impetus for all of this work and all of this thought is two-fold. First, synthetic chemistry is valuble as a central science that impacts the interfacing fields of biology, medicine, physics and polymers, and therefore the advances in chemistry that accrue from pure science endeavors in synthesis are inherently, if unpredictably, worthwhile. Second, the phenotypic effects of the isocyanoterpenes remain poorly understood, and therefore 'on-demand' access to these molecules and their analogs holds the potential to unlock the mysteries of their activity. This activity is the subject of the next section.

4 Biological Activity

4.1 Cytotoxicity and Antibacterial Activity

Given their long history, a variety of phenotypic effects have been ascribed to the marine isocyanoterpenes. Notably, these compounds generally display only moderate to weak activity in mammalian cell cytotoxicity assays (10–100 μM against a range of cell lines). In related work, early toxicity studies found that several simple isonitriles show little toxicity with oral and subcutaneous doses of up to 5 g/kg tolerated in rodent studies.[cxlv] Together these findings suggest that ICTs are generally tolerated by mammalian systems. In regard to potential therapeutic applications, early findings showed that kalihinols display moderate antibacterial activity,[cxlvi] and it was recently demonstrated that several kalihinols inhibit bacterial folate biosynthesis and show potent growth inhibition against *B. subtilis*.[cxlvii] Also adding to the range of associated biological activities, a recent report suggested that several amphilectane isonitriles have promising activity in an anti-inflammatory assay measuring thromboxane B2 and superoxide anion generation from LPS-activated rat brain microglia.[cxlviii]

4.2 Kalihinol F Copper Chelation

The use of zebrafish embryos for drug screening is of some interest due to the significant body of literature linking phenotypic observations to genotypic information in this rapidly employed animal model. A recent screen using this approach identified kalihinol F, **286**, as inducing a dramatic alteration in zebrafish development.[cxlix] The observed phenotype was similar to that observed with mutation of the copper transporter, *atp7a* – a loss of function event that induces copper deficiency. It is proposed that kalihinol F chelates copper through a bi- or tridentate chelation model (Figure 33). Support for this hypothesis included NMR studies demonstrating copper binding by the isonitrile and abolition of its zebrafish developmental phenotype with additional copper(I). Copper chelation is useful in certain therapeutic contexts, such as Wilson's disease, which result from copper accumulation. The

capacity of kalihinol F to alleviate the effects of copper toxicity was demonstrated in zebrafish and mammalian cell studies, with activity comparable to ethylenediaminetetraacetic acid (EDTA). The generality of this free-metal binding hypothesis is discussed below.

4.3 Anti-Malarial Activity

The distinguishing biological effects discovered for ICTs are their antimalarial activities. Approximately 50% of the world's population lives in regions affected by endemic malaria. Recently, malaria parasites resistant to the standard-of-care artemisinin combinations therapies (ACTs) have been found in the Thai-Cambodia border region, a common hotspot for the development and proliferation of drug-resistant parasite strains.[cl,cli,clii,cliii] The rise of artemisinin drug resistance points to significant need for novel chemotypes to act as lead compounds for further medicinal chemistry optimization, and has stimulated renewed interest in the isocyanoterpenes.[lix,lxv]

Here we attempt to provide perspective on the existing literature in this area, which has not been surveyed in a systematic fashion since an early review almost 20 years ago.[cliv] In the mid 1990's, Wright and coworkers reported that several sesquiterpene and amphilectene natural products, including **4** and **7** (Figure 1), display potent nanomolar activity against the causative agent of deadly forms of malaria *Plasmodium falciparum*. Here, as in most of these studies, activity was determined using the standard blood borne parasite growth assay [^{3}H]-hypoxanthine incorporation assay, which measures parasitic nucleic acid synthesis.[clv, clvi] A subsequent report by Miyaoka disclosed similar activity for kalihinol A.[clvii] In total, 9 reports have ascribed varying levels of anti-malarial activity–from weak to single-digit nanomolar–for isonitrile containing natural products and synthetic variants, clearly establishing the ICTs as intriguing antimalarial lead structures.[xxx,cxliii,clviii,clix,clx,clxi,clxii], A promising aspect of this collection of data is that several independent studies observed essentially equipotent activity between common chloroquine-sensitive (including D6 and HB3) and drug-resistant/ drug-insensitive (including W2 and Dd2) strains of *P. falciparum* parasites.

4.4 Anti-Malarial Activity – Mechanistic Questions

While elucidating the mechanistic basis of many antimalarial drugs has proved to be a complex matter, small molecule-heme interactions are invoked with many of the most common antimalarial drugs, including chloroquine (**289**), mefloquine, and artemisinin (**288**).[clxiii] Parasites in the intraerythrocytic stage catabolize large quantities of hemoglobin within a specialized organelle, the digestive vacuole (DV). Hemoglobin degradation leads to release of the ferrous heme prosthetic group, which rapidly oxidizes to generate Fe(III) protoporphyrin IX (FPIX, **287**).[clxiv] In addition to facilitating formation of reactive oxygen species, FPIX depletes the parasitic glutathione pool, interfering with redox homeostasis.[clxv,clxvi] Furthermore, the lipophilicity of FPIX is detrimental to lipid organization and membrane permeability and can destabilize interactions between cytoskeletal proteins and membranes.

Malaria parasites lack heme catabolism mechanisms, such as the heme oxygenase pathway,[clxvii] and thus have developed alternative routes to counter toxic effects. The putative mechanism to mitigating heme toxicity is biocrystallization of FPIX to form hemozoin. The proposed mode of action for many antimalarial drugs is disruption of this natural detoxification pathway by forming stable complexes with one or more precrystalline forms of FPIX, thereby restoring the toxic effects described above.[clxviii] Prevailing theories are that these complexes block biocrystallization by keeping FPIX solubilized or by "capping" growing crystal faces, however certain mechanistic ambiguities remains.[clxix,clxx] Nevertheless, inhibition of hemozoin formation in the presence of quinoline drugs has been visualized in live parasites[clxxi] and this has been correlated to inhibition of parasite growth.[clxxii]

While details of the biological mechanism(s) remain to be fully determined, there has been significant effort characterizing small molecule/heme binding at the structural level. With respect to the quinolines, a diverse array of non-covalent heme-drug interactions have been suggested.[clxiii] An exemplar is the dative monomer complex (**291**), which is stabilized by coordination between the quinoline nitrogen and the iron center (Figure 34). In the case of artemisinin, a heme *C*-alkylation process to form the heme complex **290** has been characterized in detail.[clxxiii] These alkylated products occur through addition of carbon-centered radicals, which arise from reductive Fe(II) mediated cleavage of the peroxide functional group, to the accessible methine positions on the heme ring system. These heme alkylation products have been identified in living mice infected with malaria.[clxxiv] It is important to note that radicals derived from artemisinin and other antimalarial peroxides have been proposed to have roles other then heme alkylation, and a recent review has covered these issues in detail.[clxxv,clxxvi]

Heme/small molecule interactions are central to the current understanding of the ICT natural products discussed here. A variety of simple alkyl isonitriles bind the iron center of protein bound Fe(II)-heme, and starting with seminal studies by Pauling in 1956, these have served as probes of hemoglobin, myoglobin, and related proteins.[clxxvii] Variations in the strength of isonitrile-heme binding interaction have been used to interrogate steric and electronic parameters of the heme-binding site, though advances in structural biology have largely supplanted such applications. More recently, Marletta and coworkers demonstrated a role for a nitric oxide/cysteine interaction in guanylate cyclase by using *n*-butyl isonitrile to block the heme-binding site.[clxxviii]

In 2001, Wright suggested that formation of isonitrile-heme(II) complex (**292**, Figure 34) or the symmetrical binary complex lies at the heart of the antimalarial activity of these compounds. Isonitrile-heme binding was demonstrated using UV Stokes shift and ESI-MS. Several compounds, including **7**, were shown to inhibit β-hematin formation and prevent the destruction of heme by peroxide and glutathione. In addition, a computational pseudoreceptor model was proposed.[clix] Similar to the proposed mechanism of action for quinoline and peroxide drugs, the suggestion is that these complexes disrupt hemozoin formation, leading to parasite death. This single binding interaction has been cited as the origin of the antimalarial activity of isonitrile natural products.[clxxix] While appealing, single-point binding of isonitriles to Fe(II)-heme does not offer a clear explanation for the complex

SAR patterns highlighted above, nor does it explain the 200 nM activity of the formamide amphilectene (Figure 34). In other words, the binding of heme by ICTs may be only one mechanism that contributes to their potency amidst possibly broad polypharmacology. In fact, recent studies examining chloroquine and related quinolines have suggested that targets other than heme are likely important for the cytocidal activity for these long-studied and extensively-used agents.[clxxx]

Given the capacity of isonitriles to form transition metal complexes, a role for free metal binding is conceivable. The isolation the isonitrile-copper complex, **91** (Figure 5d), and the zebrafish study by Ireland, both discussed above, provide support for this notion. Furthermore, anti-malarial activity has been reported for several copper and iron chelating scaffolds. In particular, iron chelators have been studied extensively, and desferrioxamine has undergone clinical studies to treat malaria with significant success.[clxxxi] With regard to copper chelation, a cell permeable copper-chelating compound was shown to arrest parasite growth at the ring-to-trophozoite stage transition.[clxxxii] The antimalarial activity of these agents is proposed to occur by two mechanisms: sequestration of free metal ions required for metabolic pathways and through the formation of toxic metal complexes.

The structure-activity relationships that can be defined from existing data suggest that ambiguities remain regarding the mechanism of action (Figure 35 and 36). The following general observations can be made: (1) the presence of the isonitrile is required and, with one particularly notable exception (discussed below), only the isonitrile, not the corresponding isothiocyanate or formamide, display high activity. (2) The presence of an isonitrile alone is not sufficient for potent activity. For example, in a study examining the activity of 10 simple non-natural isonitriles, only 4 showed even modest activity (MIC = 3–14 μM). In these efforts, adamantyl isonitrile, **293**, was the most active (MIC = 2.5 mM) and was shown to display weak *in vivo* activity (2 of 5 mice survived to day 28 at 50 mg/kg/d administration) with poor therapeutic index (toxic at day 4 at 100 mg/kg/d).[clx] (3) In several cases, functionality distal to the isonitrile is critical to activity. Comparison of **294** and **9** suggests that a distal stereocenter affects the potency by over 20 fold, which is difficult to explain based on a non-specific (non-lock-and-key) binding event (Figure 35). In a notable exception to these rules, the formamide of the mono-isonitrile amphilectane is reported to exhibit quite potent activity (200 nM, Figure 36).[clxii] In total, these results provide conflicting information about activity: if free-heme or free-metal binding is key, then why is stereochemistry important for potency? Alternatively, if the observations relate to drastic differences in pharmacokinetics (membrane permeability, efflux, metabolic degradation, etc.), why is formamide **295** active? In particular, protein active site binding is a distinct possibility that has not been examined in detail to date. A full understanding of the reported anti-malarial activity will certainly require further study.

5 Conclusion

We conclude with several points that emerge from the discussion above. First, although the isocyanoterpenes are recognizable by the substitution patterns imparted by their biosynthesis (Part 1), the structural diversity within this class has thwarted attempts to unify their chemical synthesis, particularly with a high level of efficiency (Part 2). Second, the value of

synthetic chemistry research within this class is two-fold: as stimulus for the development of new chemical methods (Part 2), and as a means to procure material for biological study (Part 3). Third, we note that research efforts to broadly and rigorously address molecular interactions of this class in complex biological settings (Part 3) are noticeably absent, perhaps due to the assumption (correct or incorrect) that these molecules possess nonspecific mechanisms of action. The recent synthetic advances highlighted here, coupled with progress in chemoproteomic techniques suggest more rigorous evaluation of the ICTs as a potentially intriguing avenue for future work. The chemical reactivity of isonitriles (Part 1, Figure 1C) may underpin target interactions and should be considered as these efforts proceed.

Acknowledgements

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Figure 1.
(A) Isonitrile-Containing Natural Products (B) General Biosynthetic Scheme (C) Representative Isonitrile Reactivity.
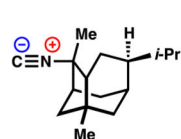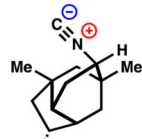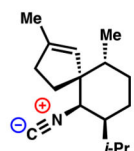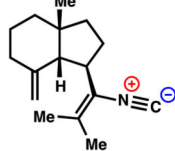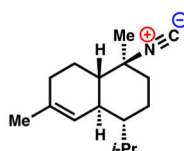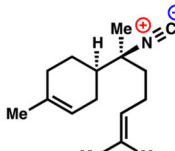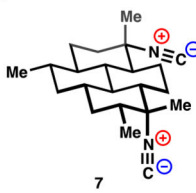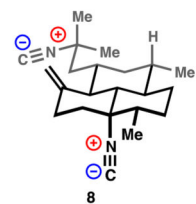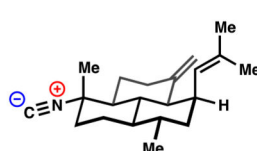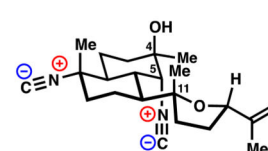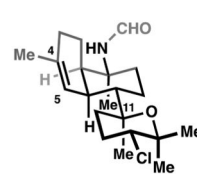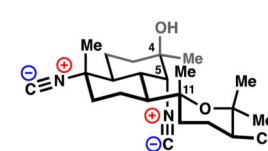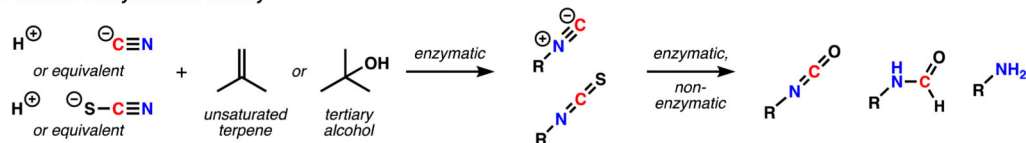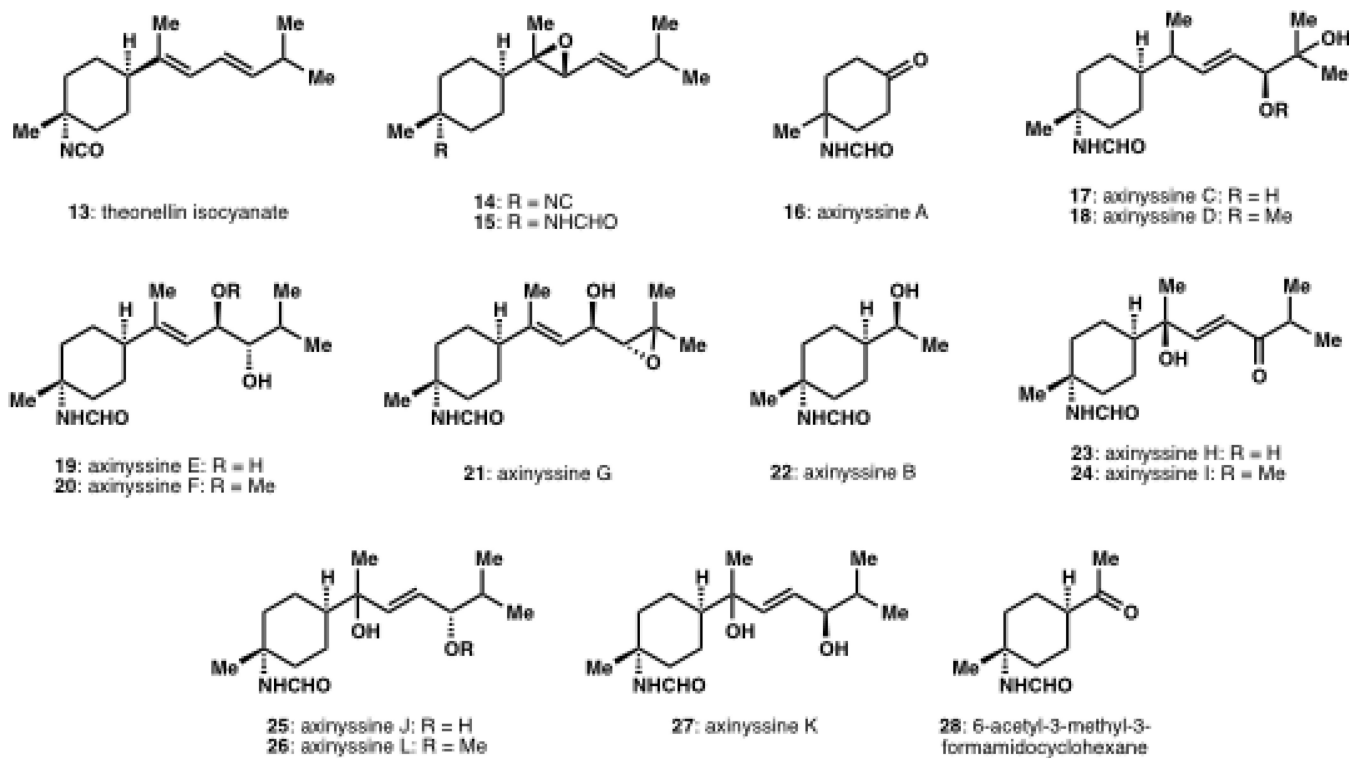
Figure 2.
Bisabolene sesquiterpenes isolated from 2004–2014.

29: 3-oxo-axisonitrile

30: axinynitrile

31: axinisothiocyanate A

32: axinisothiocyanate B,
R¹ = NCS, R² = Me
33: axinisothiocyanate C,
R¹ = Me, R² = NCS

34: axinisothiocyanate D,
R¹ = Me, R² = OH
35: axinisothiocyanate E,
R¹ = OH, R² = Me

36: axinisothiocyanate F

37: axinisothiocyanate G

38: axinisothiocyanate H,
R¹ = OOH, R² = i-Pr
39: axinisothiocyanate I,
R¹ = i-Pr, R² = OOH
40: axinisothiocyanate J,
R¹ = i-Pr, R² = H

41: axinisothiocyanate K

42: axinisothiocyanate L

43: axinisothiocyanate M,
R = OH
44: axinisothiocyanate N,
R = OOH

45

46

47

48: axisocyanate-3

Figure 3.
Sesquiterpenes isolated from 2004–2014.

Caverenes and kalihinols

49: caverene A   **50**: caverene B   **51**: caverene C   **52**: caverene D   **53**: kalihinene E

54: kalihinene F   **55**: kalihipyran   **56**: 15-formamido kalihinol F   **57**: 10-formamido kalihinol F   **58**: 10-epi-kalihinol X

59: kalihinol M   **60**: kalihinol N   **61**: kalihinol O:
R¹ = NCS, R² = NC
62: kalihinol Q:
R¹ = NC, R² = NCS
63: kalihinol R:
R¹ = NCS, R² = NCS
    64: kalihinol P:
R¹ = NC, R² = NCS
65: kalihinol S:
R¹ = NCS, R² = NHCHO
    66: kalihinol T

Amphilectenes and cycloamphilectenes (adocianes)

67   **68**   **69**   **70**   **71**

72   **73**   **74**: R = NCO
75: R= NCS
    76   **77**: R = NHMe
78: R= NCHO

79   **80**   **81**   **82**   **83**

Figure 4.
Diterpene natural products isolated from 2004–2014.
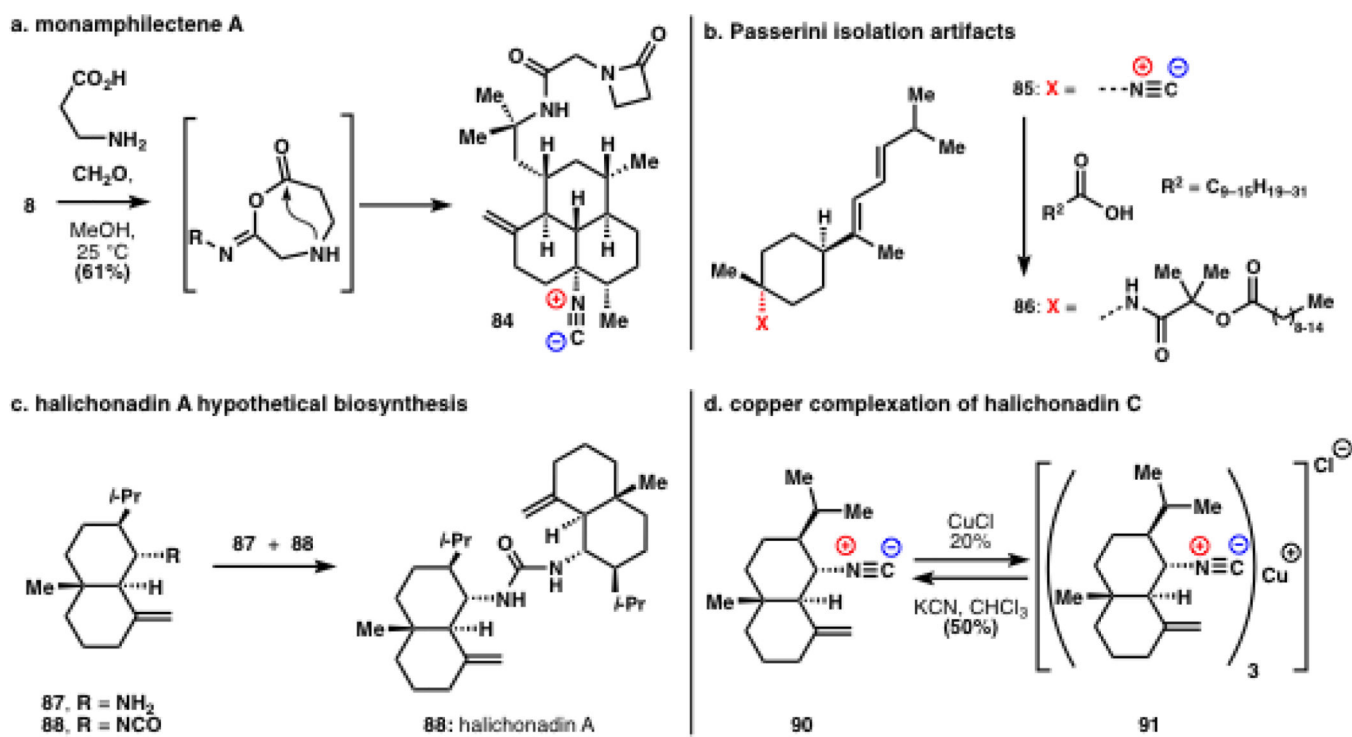
Figure 5.
a. Semisynthesis of monamphilectine A. **b.** Passerini isolation artifacts. **c.** Proposed biosynthesis of urea-linked sesquiterpenes. **d.** Copper complexation and decomplexation of isocyanoterpenes.
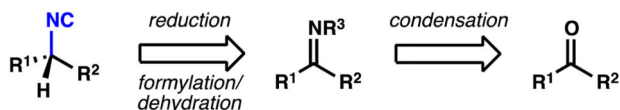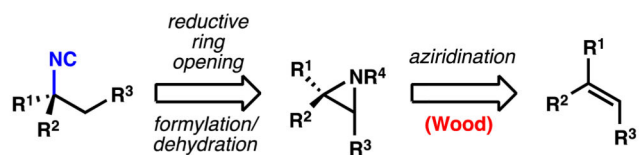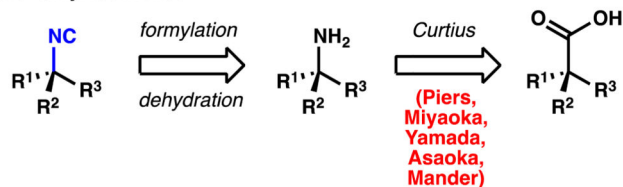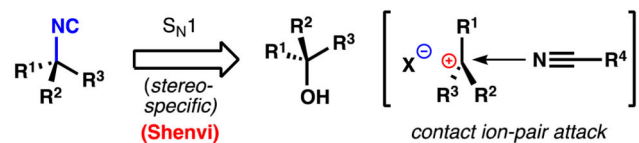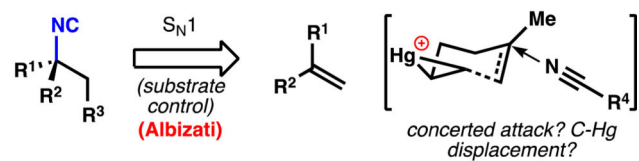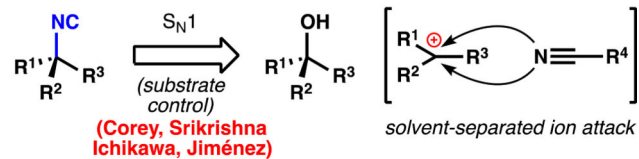
Figure 6.
Retrosynthetic strategies to generate stereogenic *sec-* and *tert*-alkyl isonitriles.
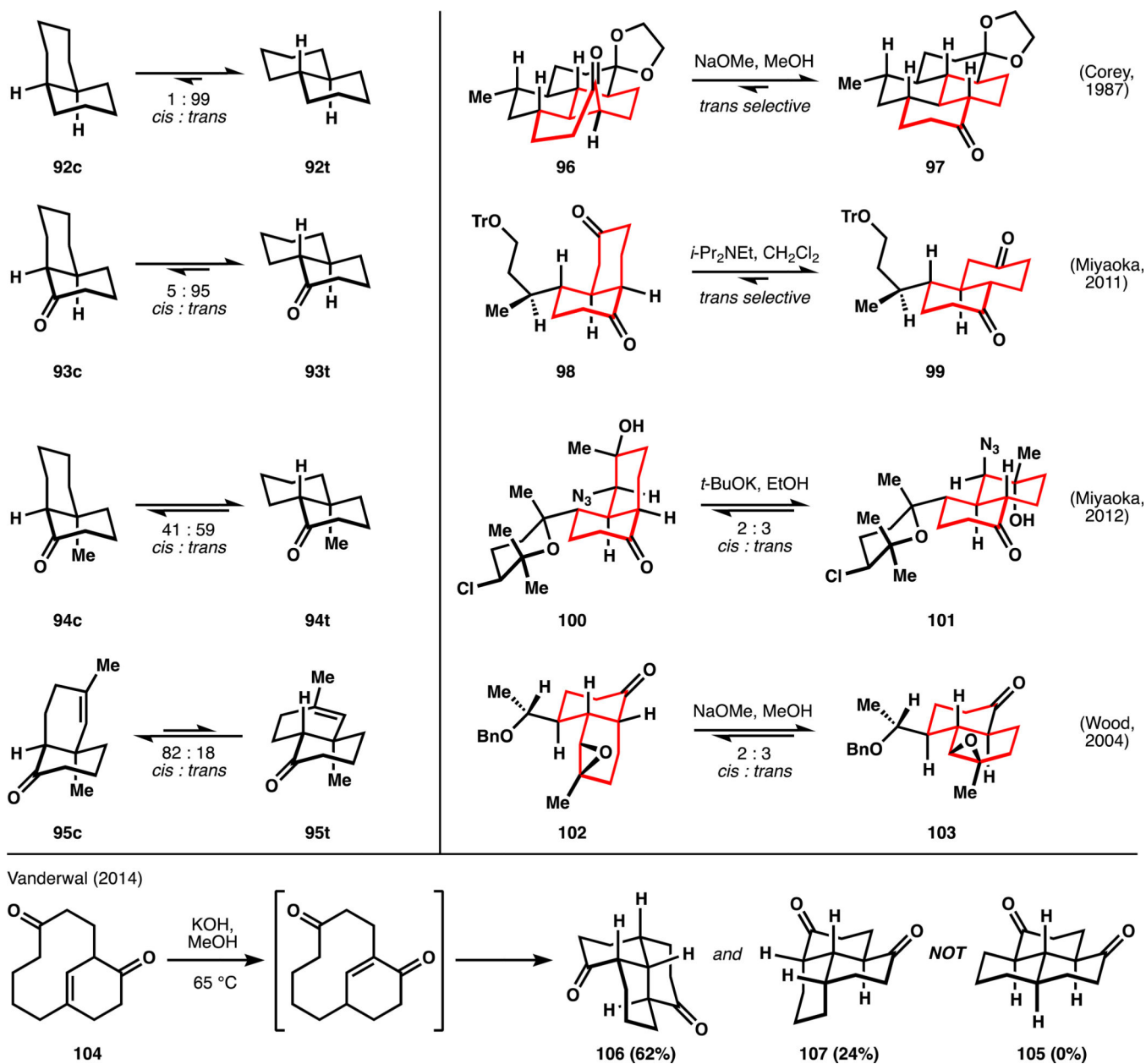
Figure 7.
Cis-/trans-isomerism of substituted decalins.
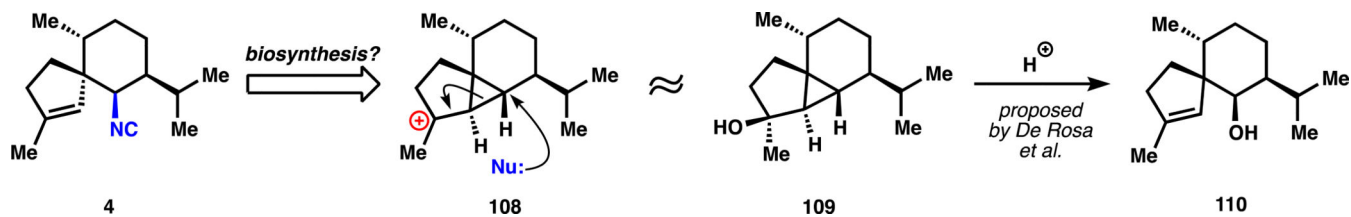
Figure 8.
A hypothetical biosynthesis of **4** based on related terpene relationships.

a. retrosynthetic analysis
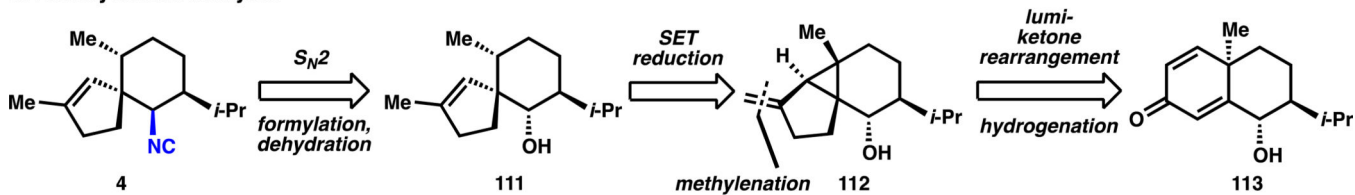
b. chemical synthesis
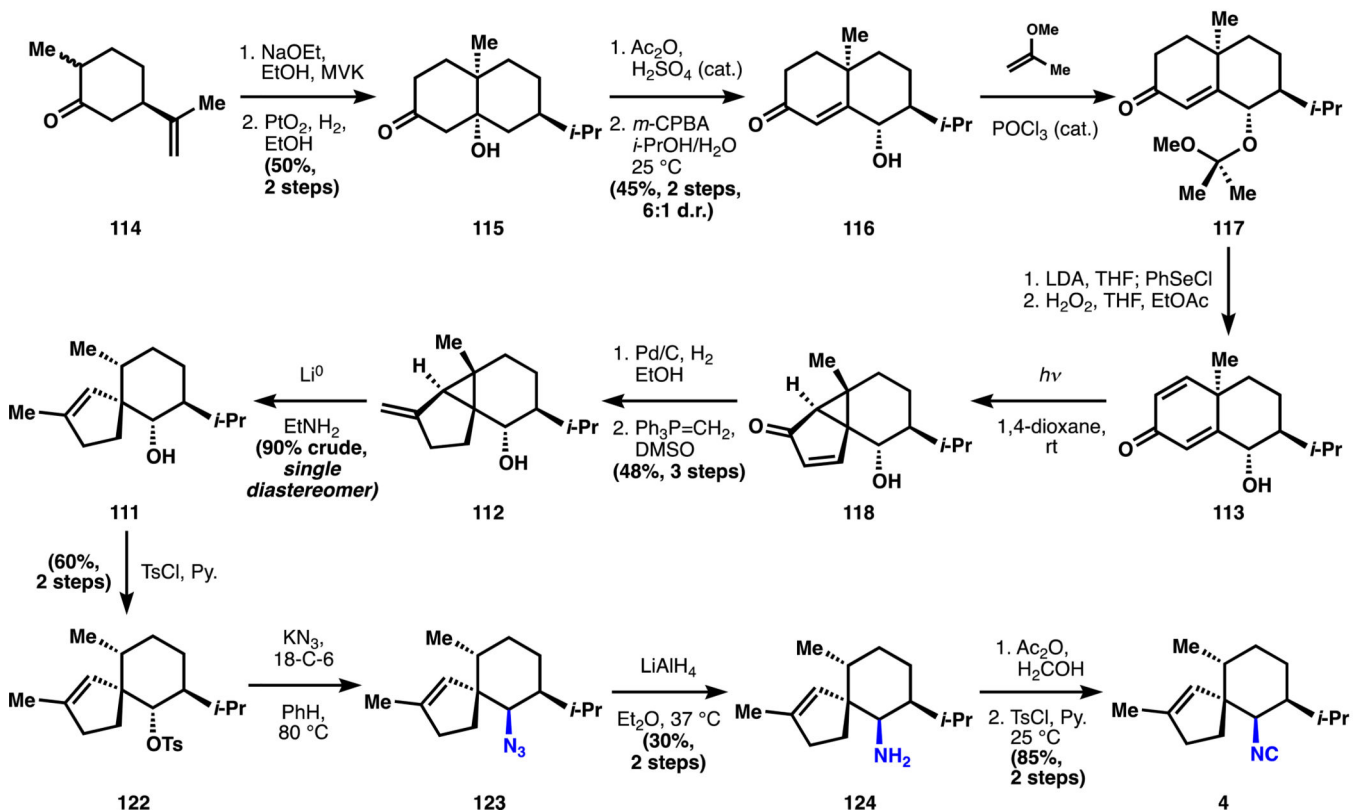
Figure 9.
Caine's synthesis of (–)-axisonitrile-3.
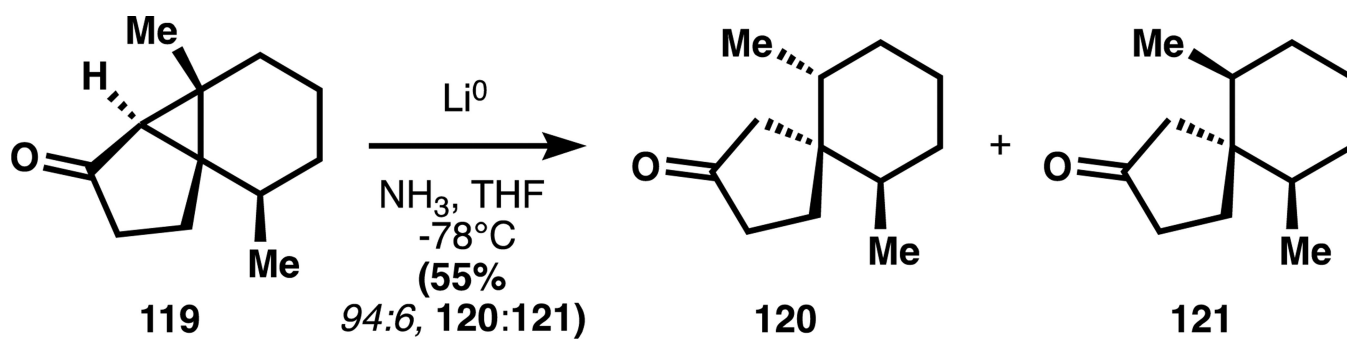
Figure 10.
Piers' studies on the stereochemistry of cyclopropyl ketone reductive cleavage.
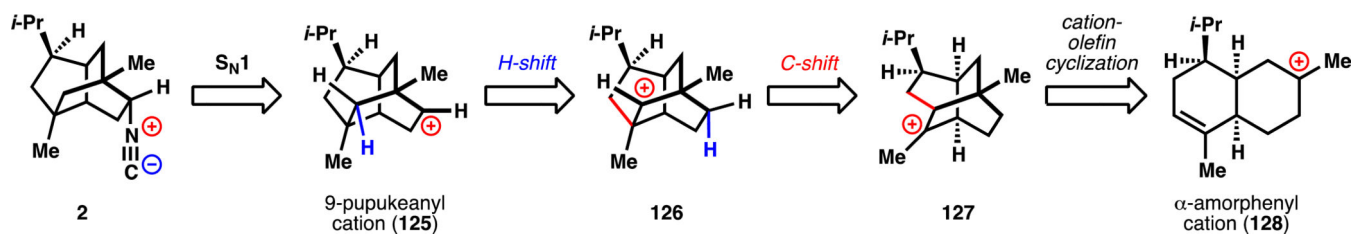
Figure 11.
Hypothesized biosynthesis of **2**.

a. retrosynthetic analysis

b. chemical synthesis
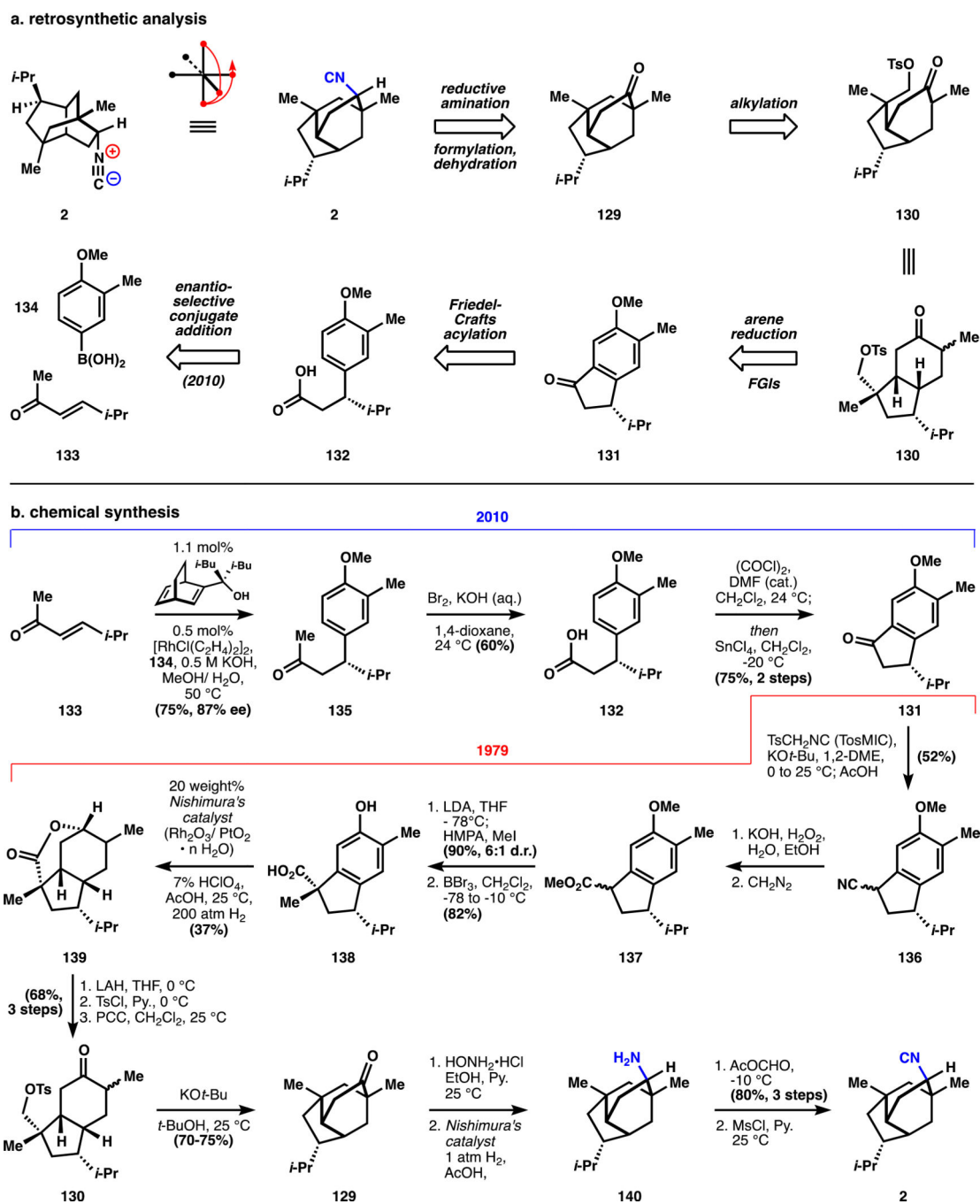
Figure 12.
a. Corey's analysis of **2**; **b.** Corey's chemical synthesis of **2**.

a. retrosynthetic analysis
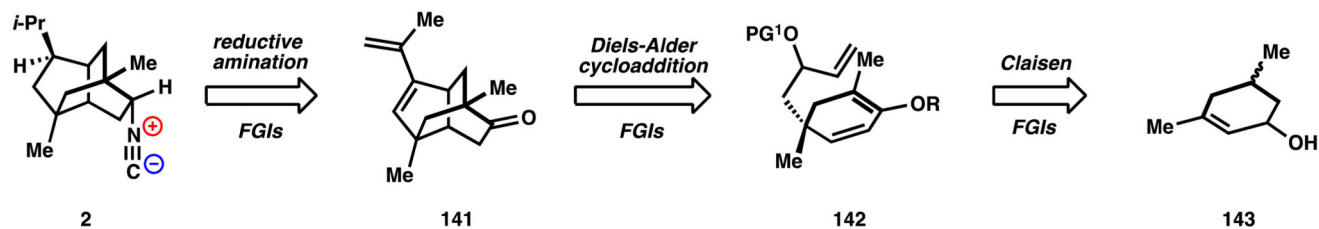
b. chemical synthesis
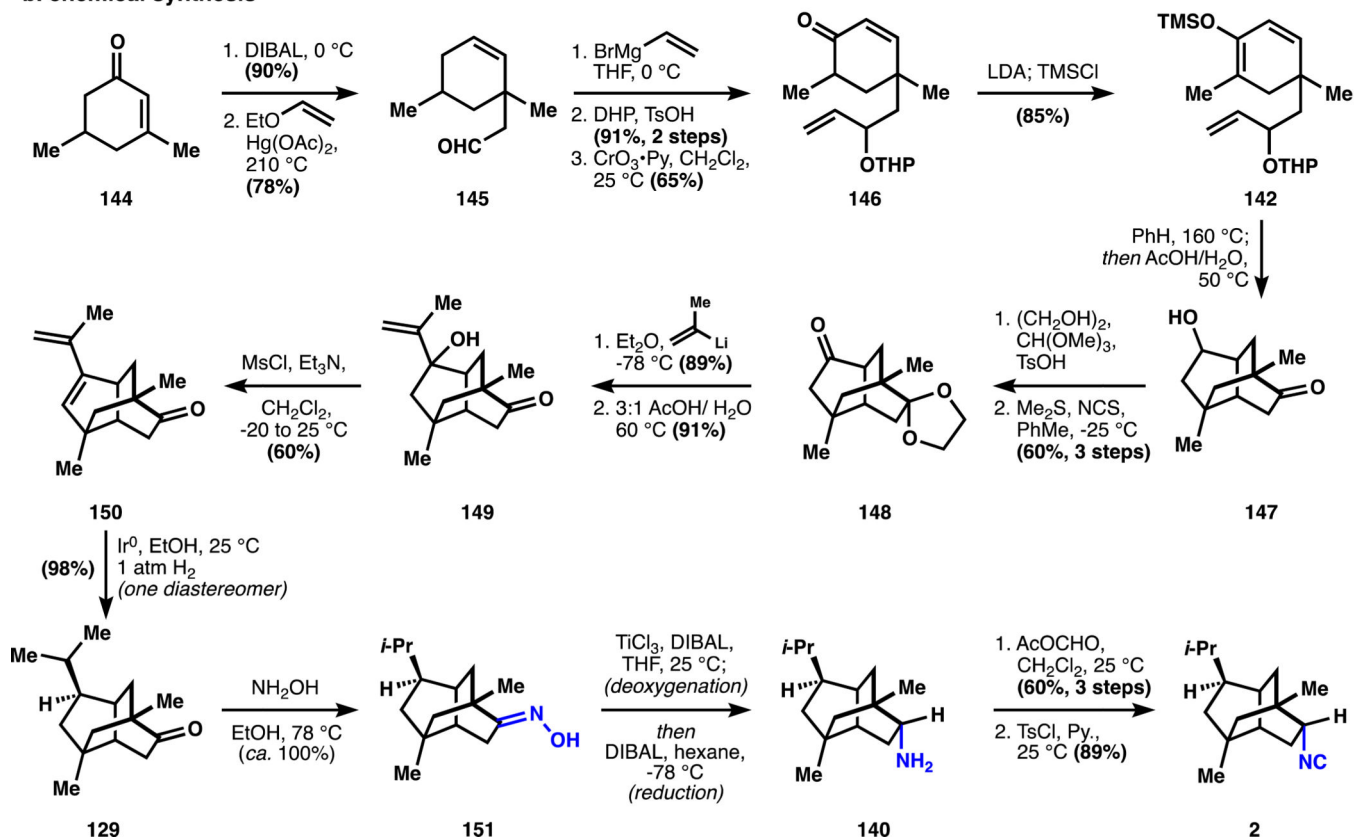
Figure 13.
a. Yamamoto's analysis of **2**. **b.** Yamamoto's chemical synthesis of **2**.

a. retrosynthetic analysis
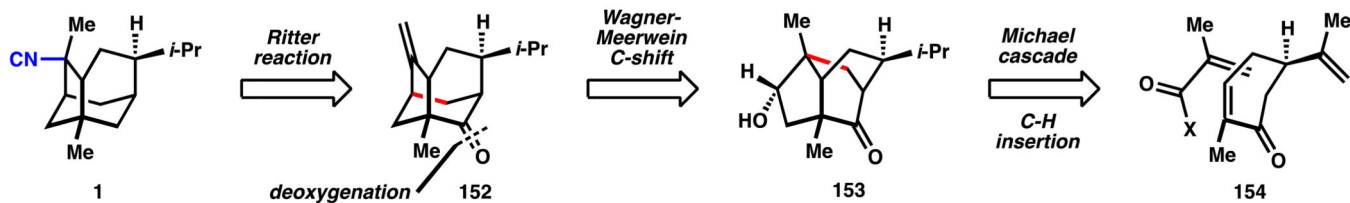
b. chemical synthesis
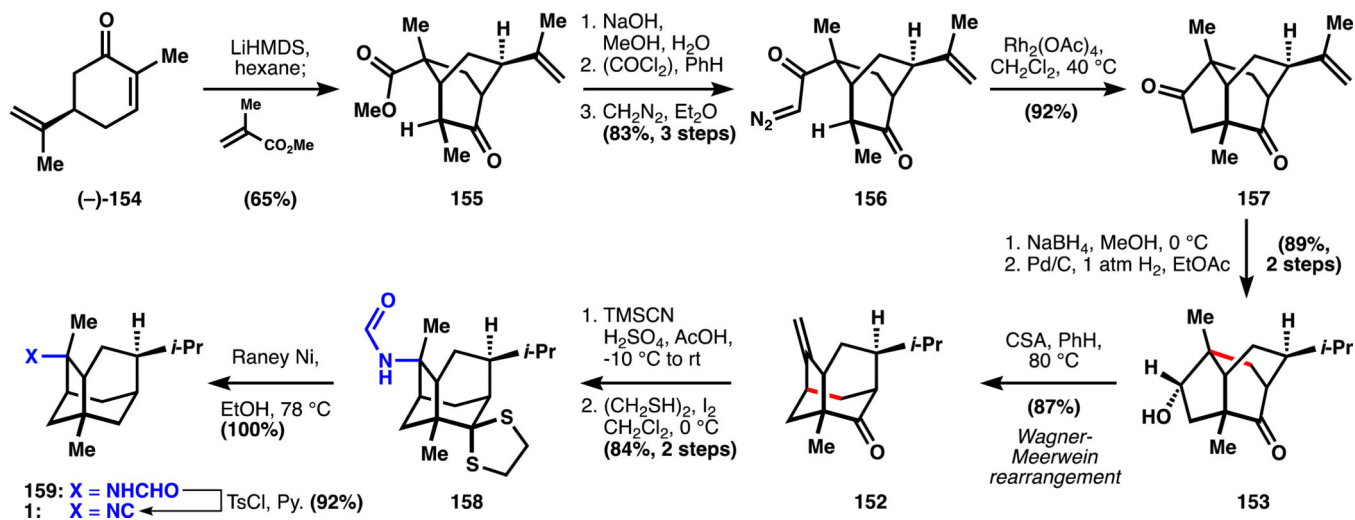
Figure 14.
a. Srikrishna's analysis of **1**; **b.** Srikrishna's chemical synthesis of **1**.
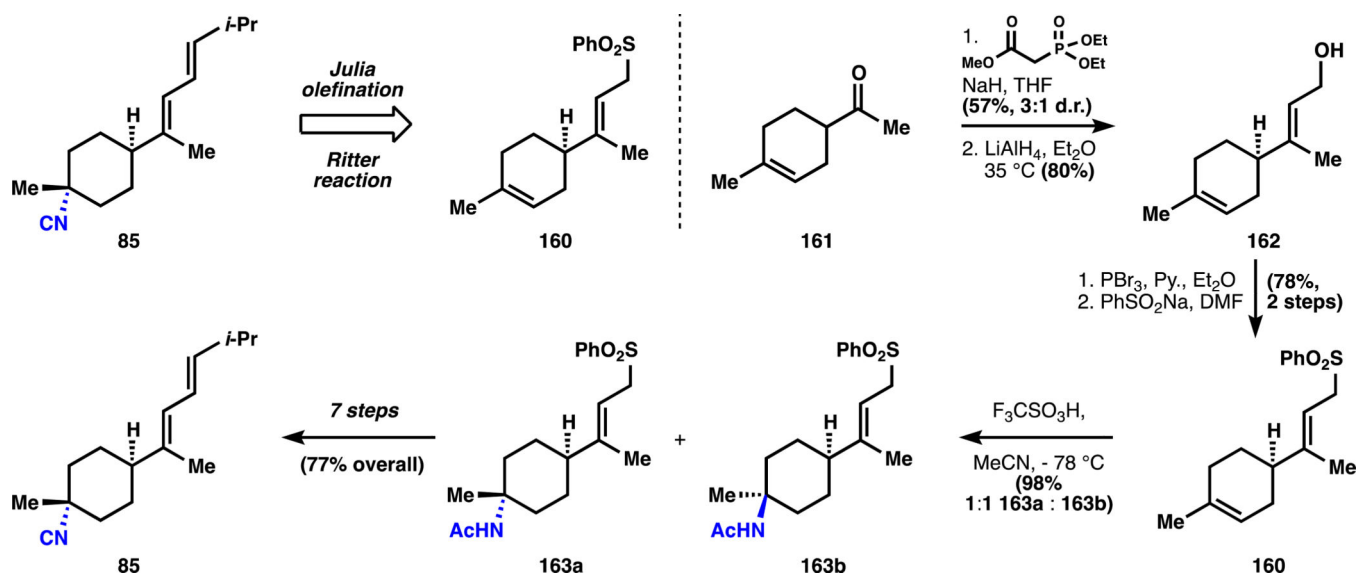
Figure 15.
Ichikawa's analysis and synthesis of **85** using a Ritter reaction.
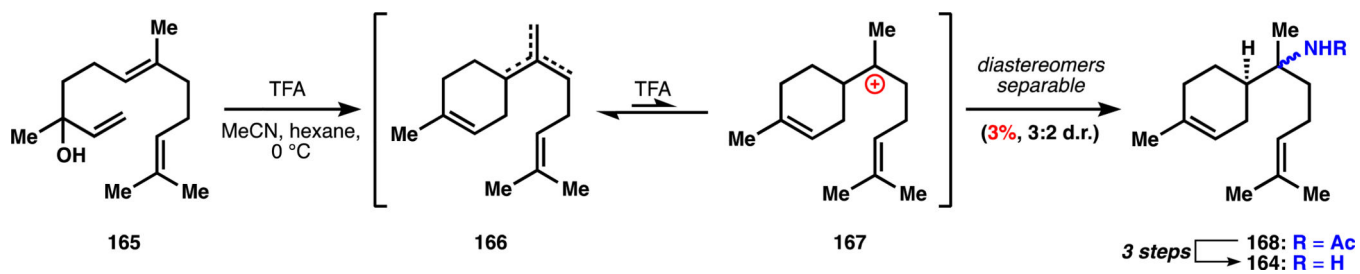
Figure 16.
Ichikawa's polycyclization of nerolidol en route to bisabolyl amine **164**.
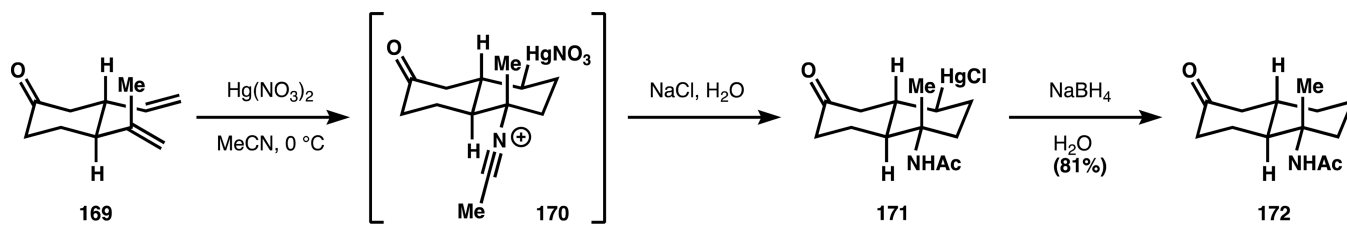
Figure 17.
Albizati's mercury-mediated polyene cyclization and nitrile capture.
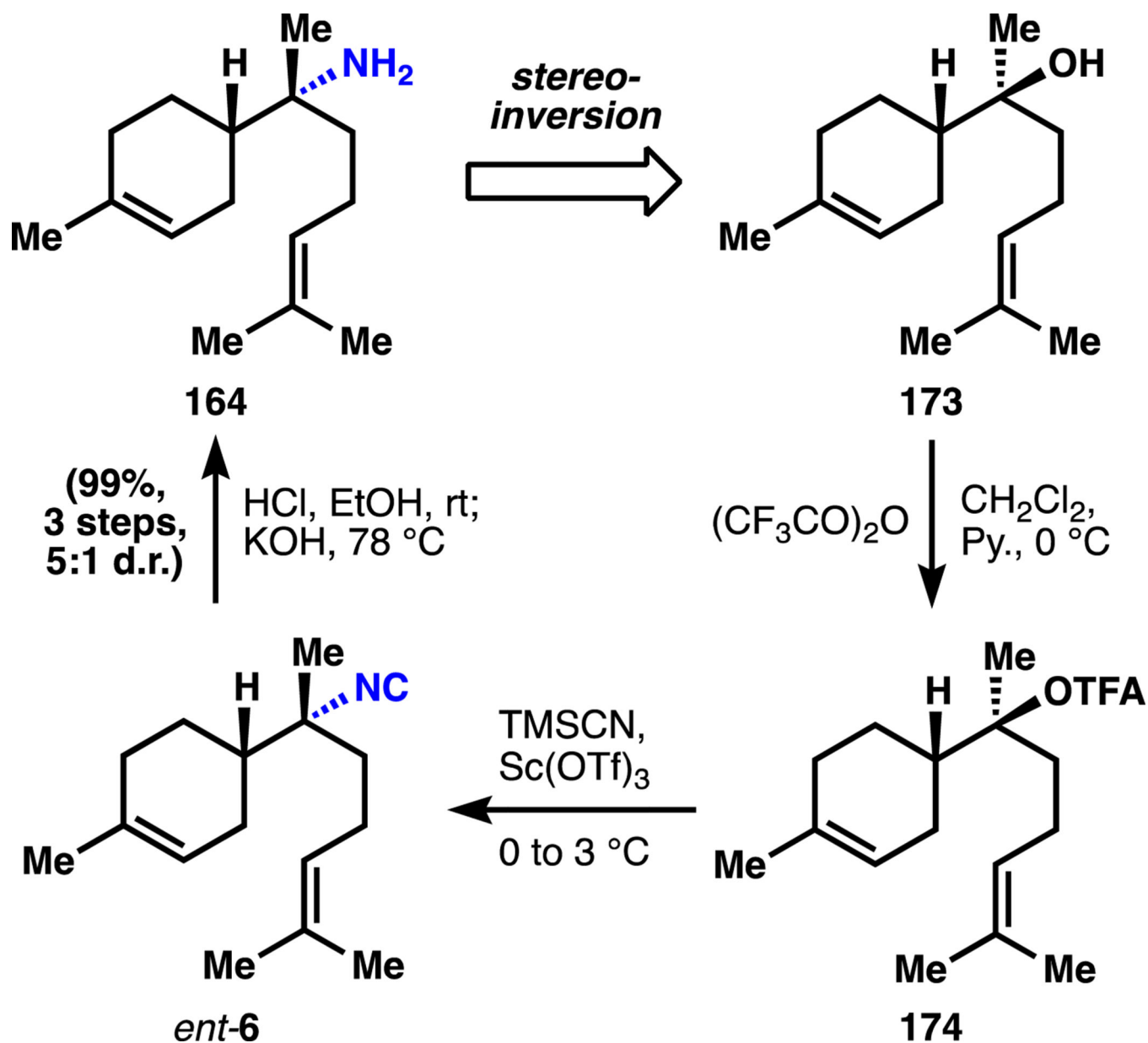
Figure 18.
Shenvi's route to (−)-**164** via stereoinversion of **173**.

a. retrosynthetic analysis
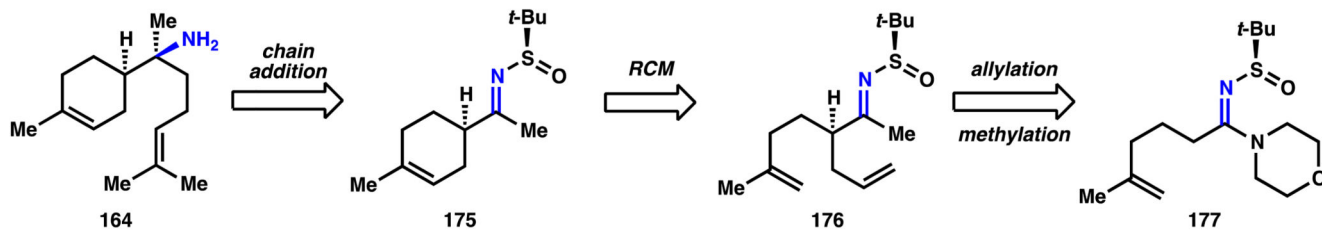
b. chemical synthesis
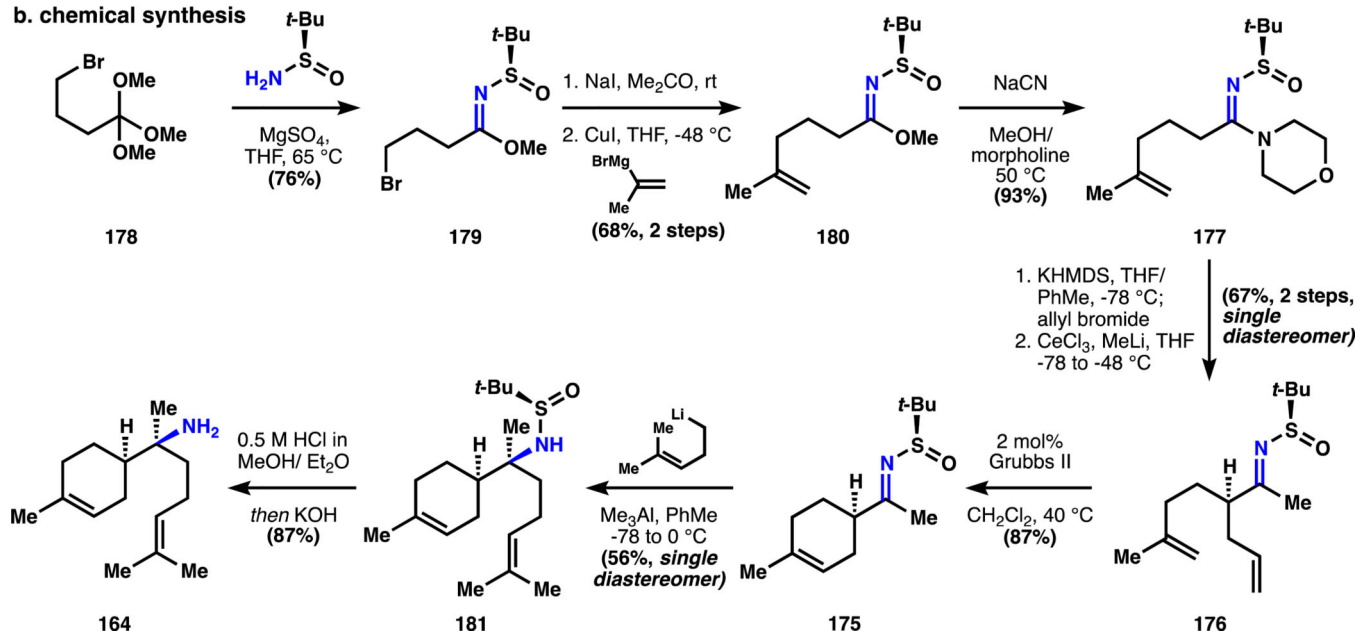
Figure 19.
a. Ellman's analysis and **b.** synthesis of (+)-**164** using his auxiliary.

7

182

8

183
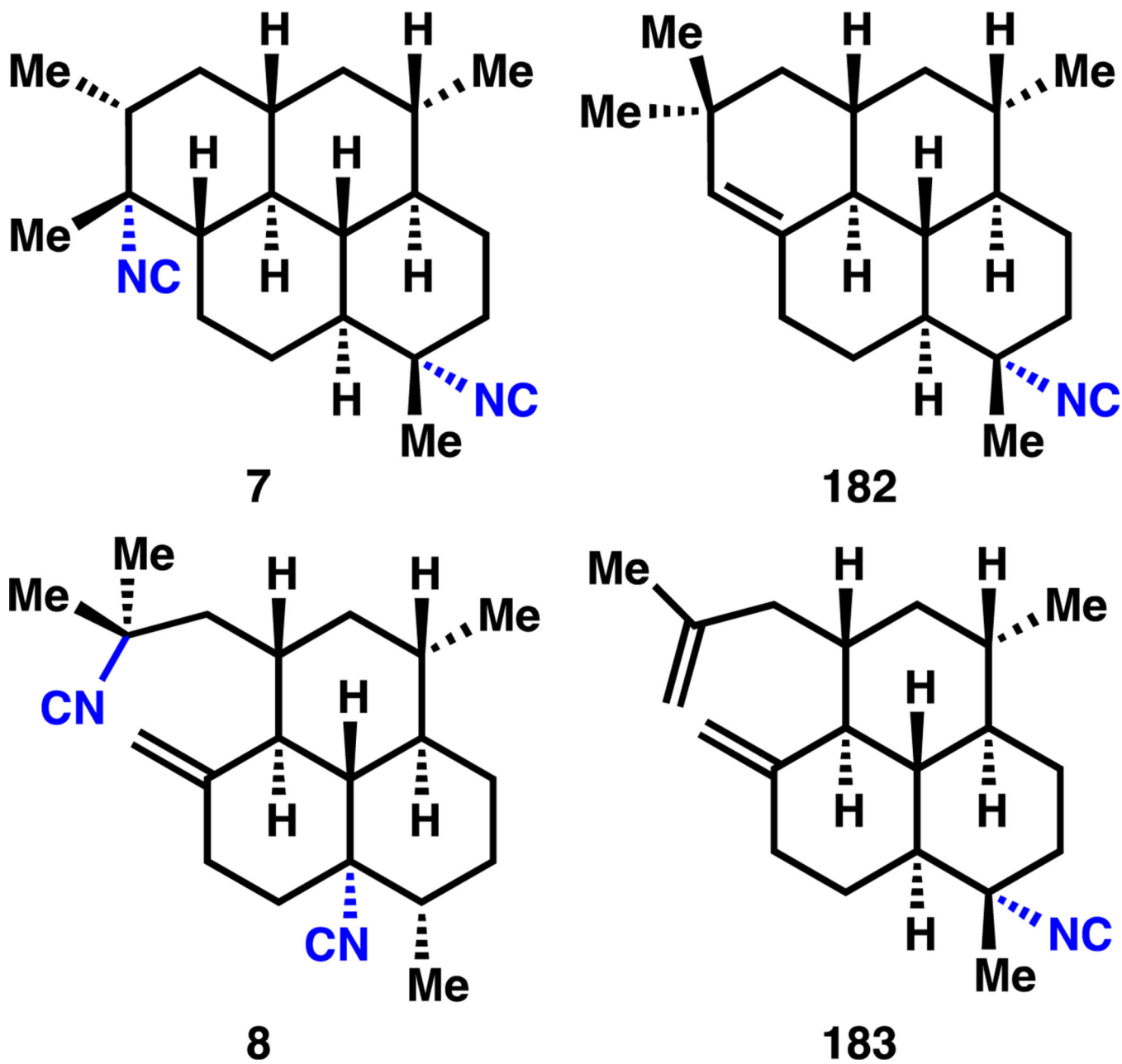
Figure 20.
Amphilectenes and adocianes.
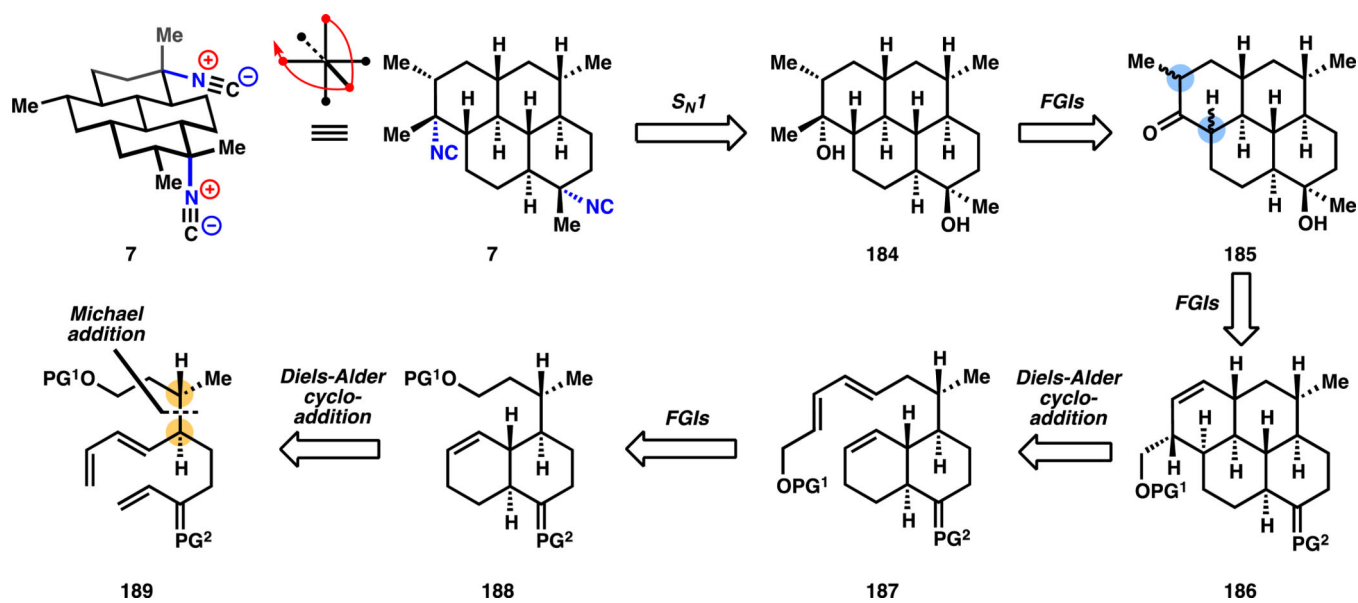
Figure 21.
Corey's retrosynthetic analysis of (+)-7,20-diisocyanoadociane (**7**).
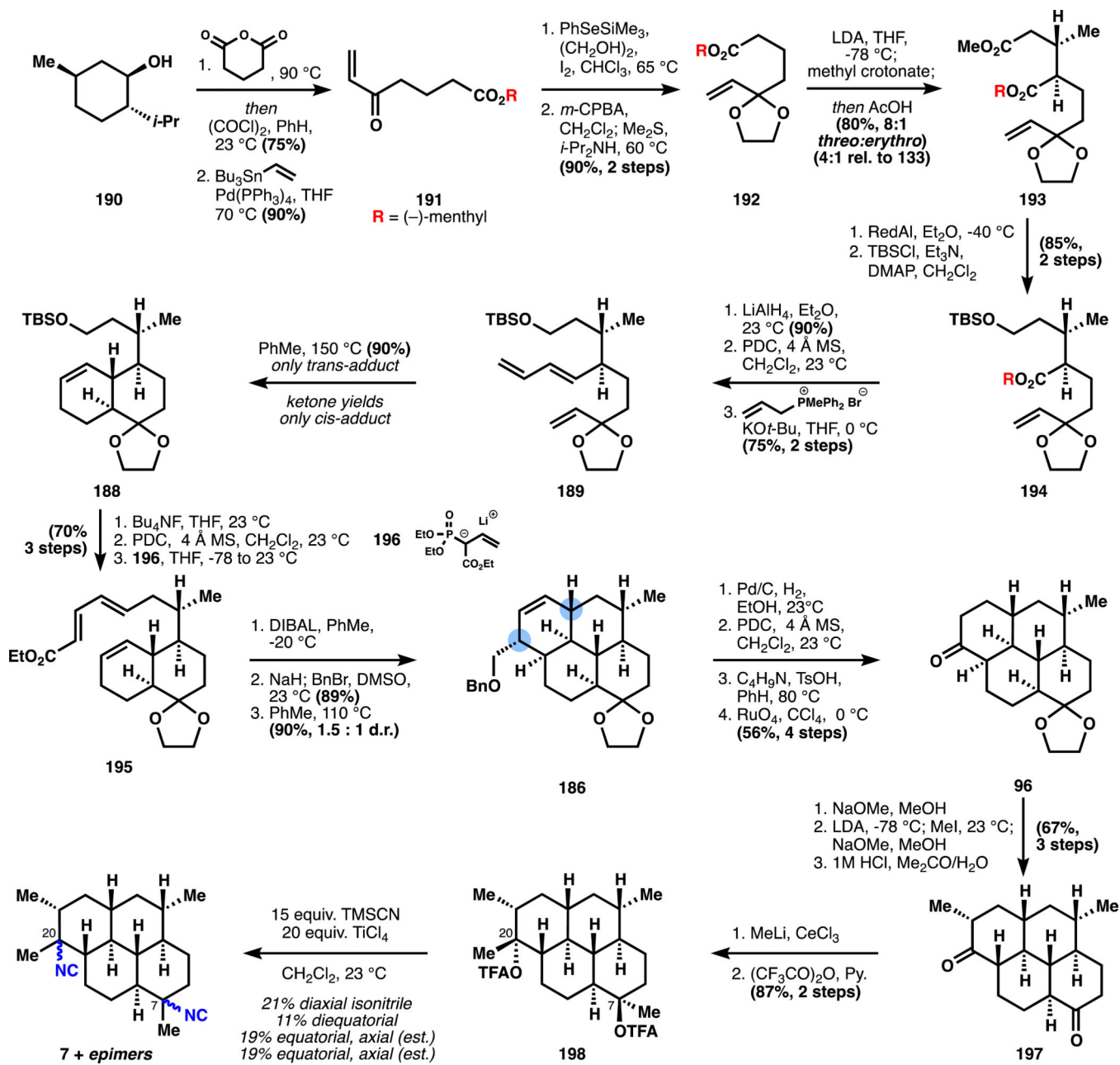
Figure 22.
Corey's chemical synthesis of **7**.
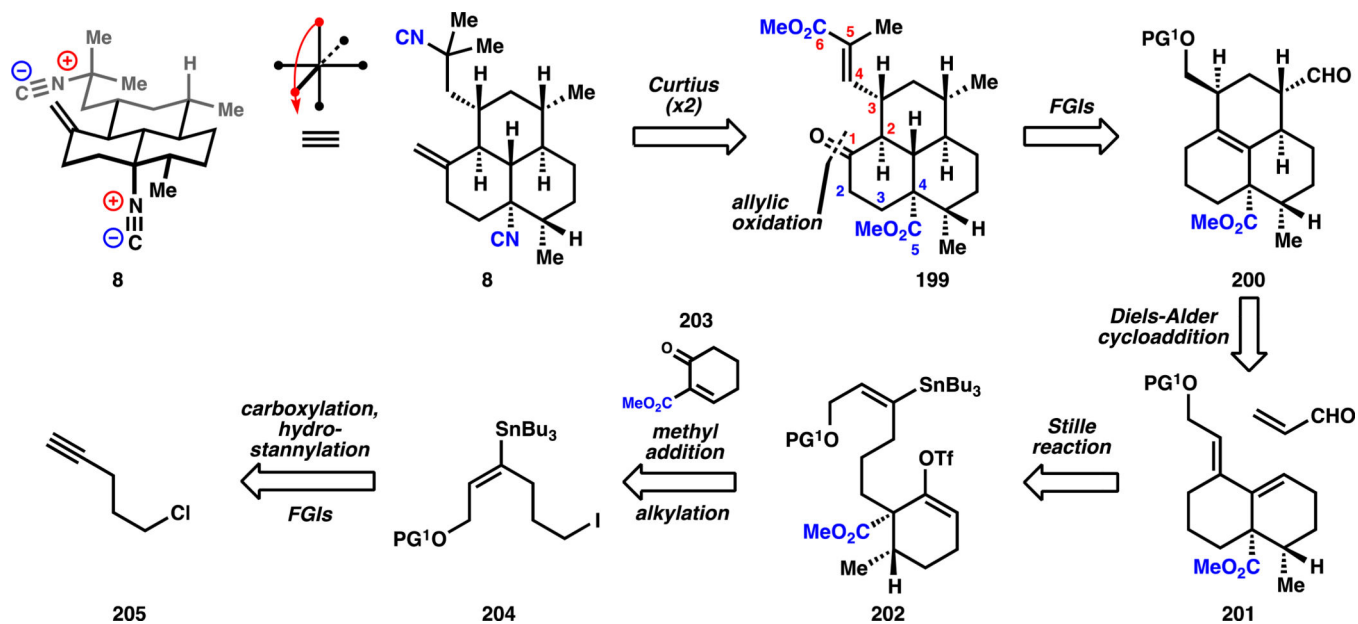
Figure 23.
Piers' analysis of **8** using difunctional reagent **204** as precursor to diene **201**.
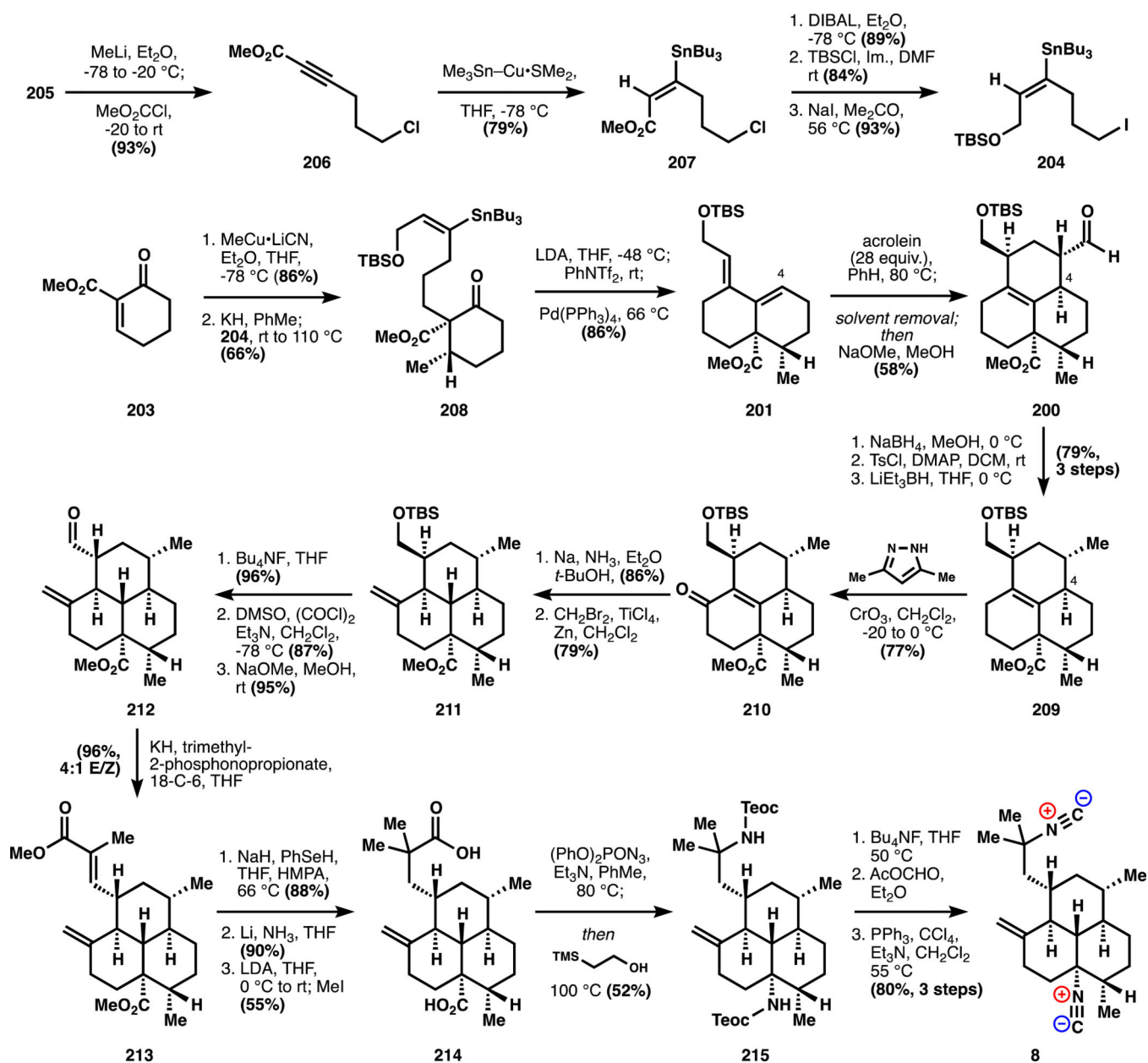
Figure 24.
Piers' synthesis of **8**.

a. retrosynthetic analysis
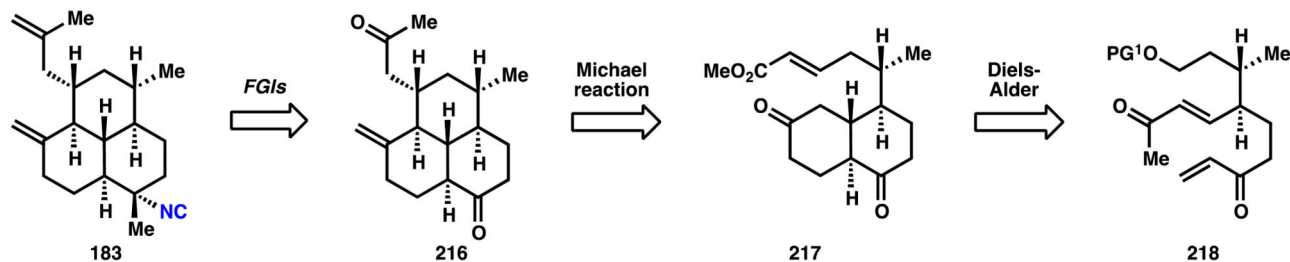
b. chemical synthesis
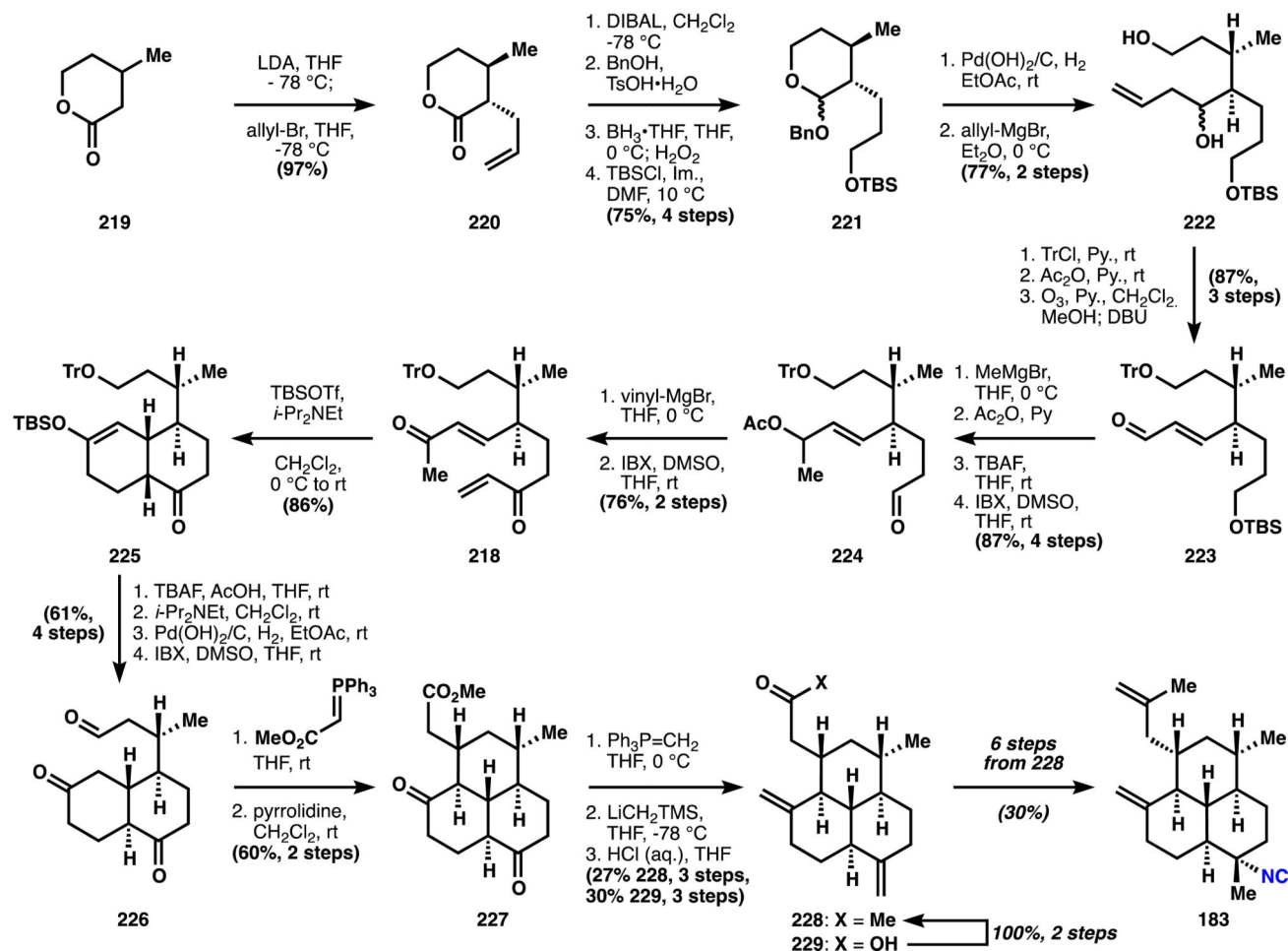
Figure 25.
a. Miyaoka's analysis and **b.** synthesis of amphilectene **183**.
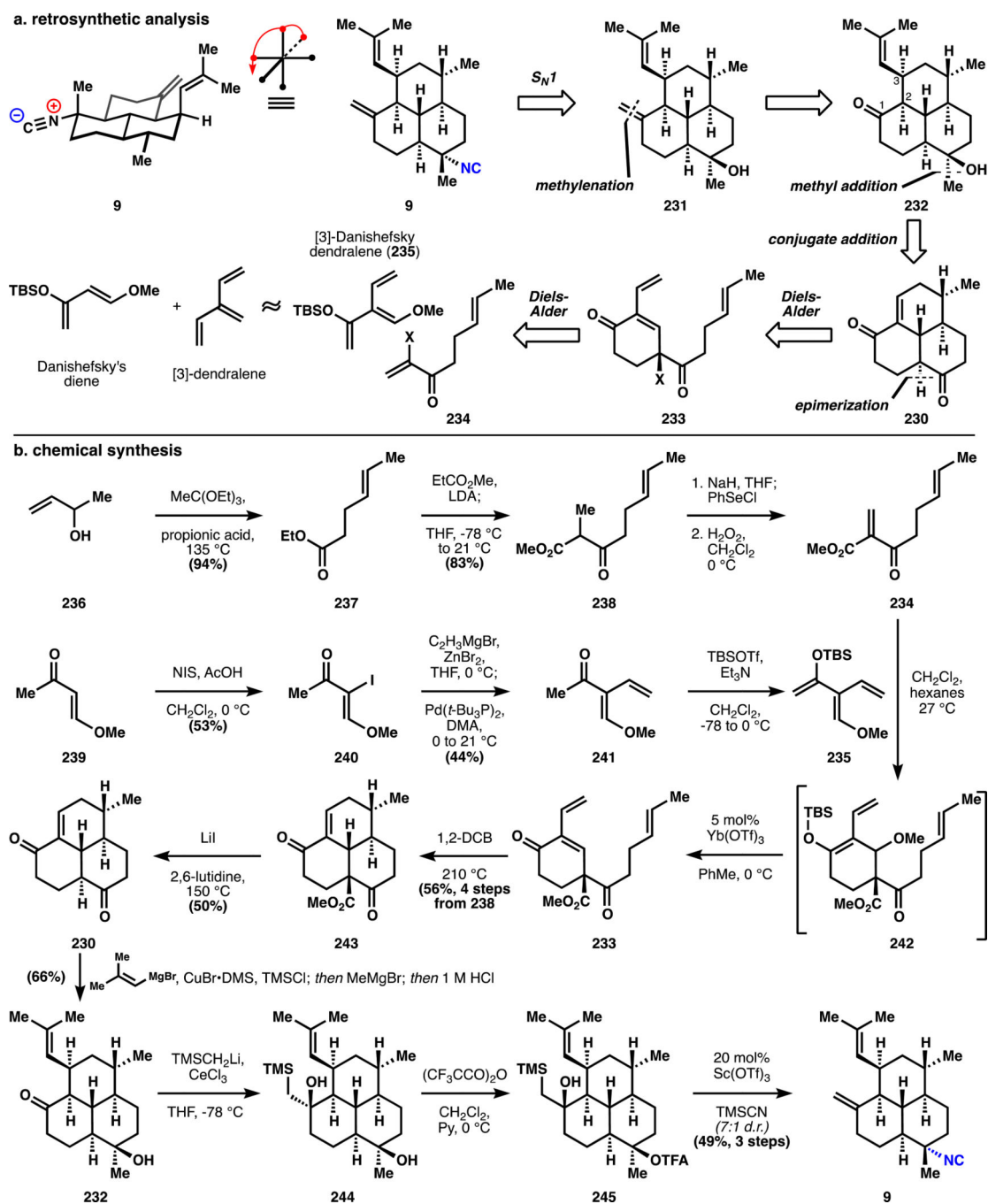
Figure 26.

a. Shenvi's analysis and **b.** synthesis of amphilectene **9** using [3]-Danishefsky dendralene **235** and a stereospecific S_N1 reaction.
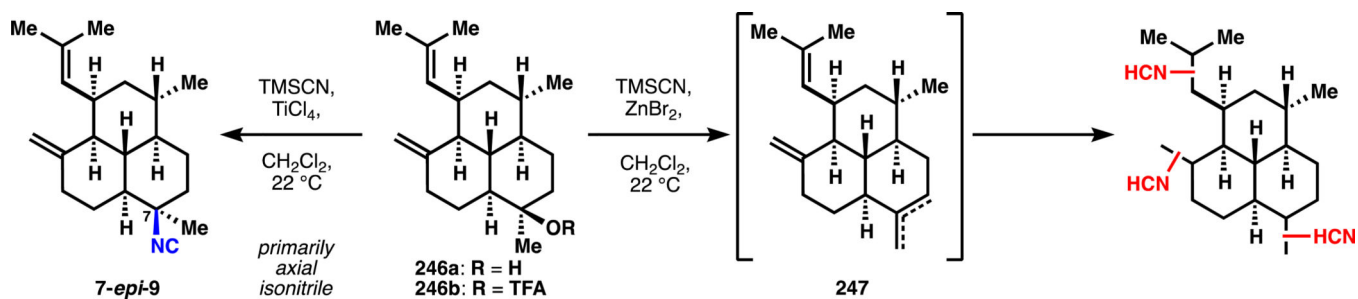
Figure 27.
Pitfalls of other methods for isocyanation in the synthesis of **7**.
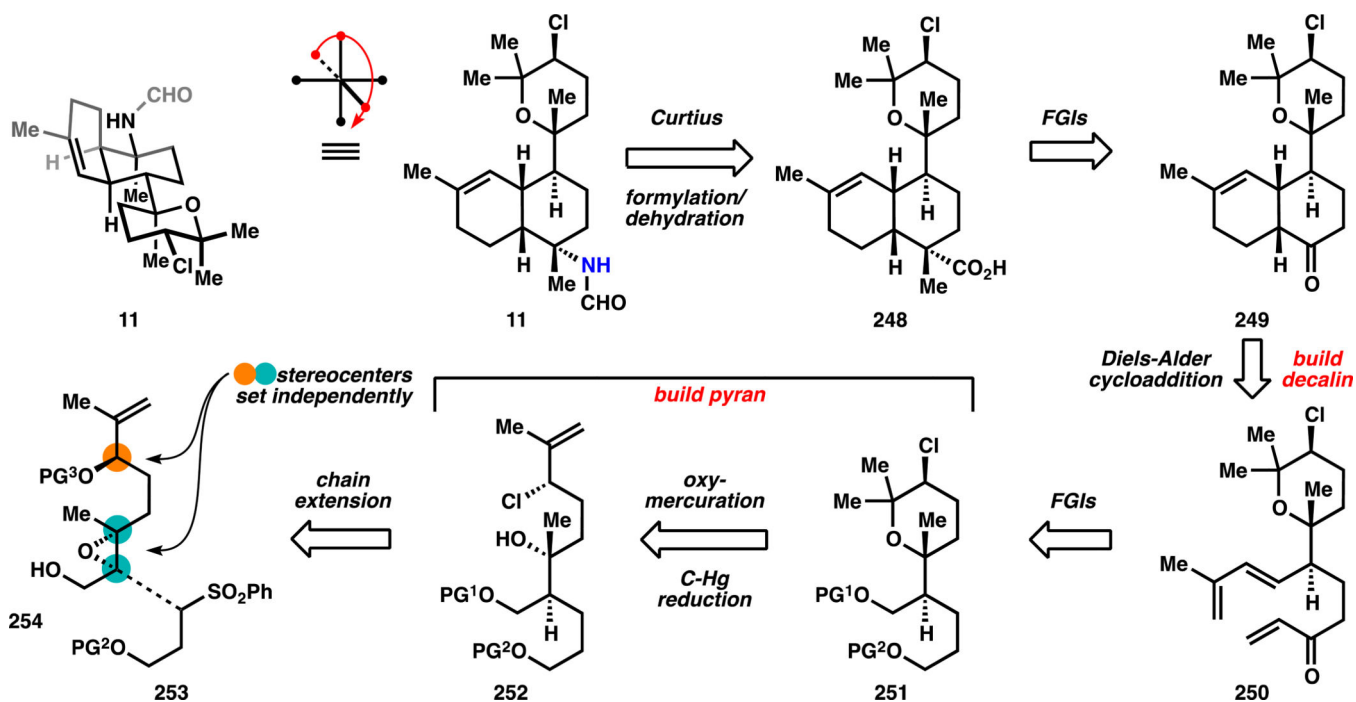
Figure 28.
Retrosynthetic analysis of **11** according to Miyaoka and Yamada's synthesis.
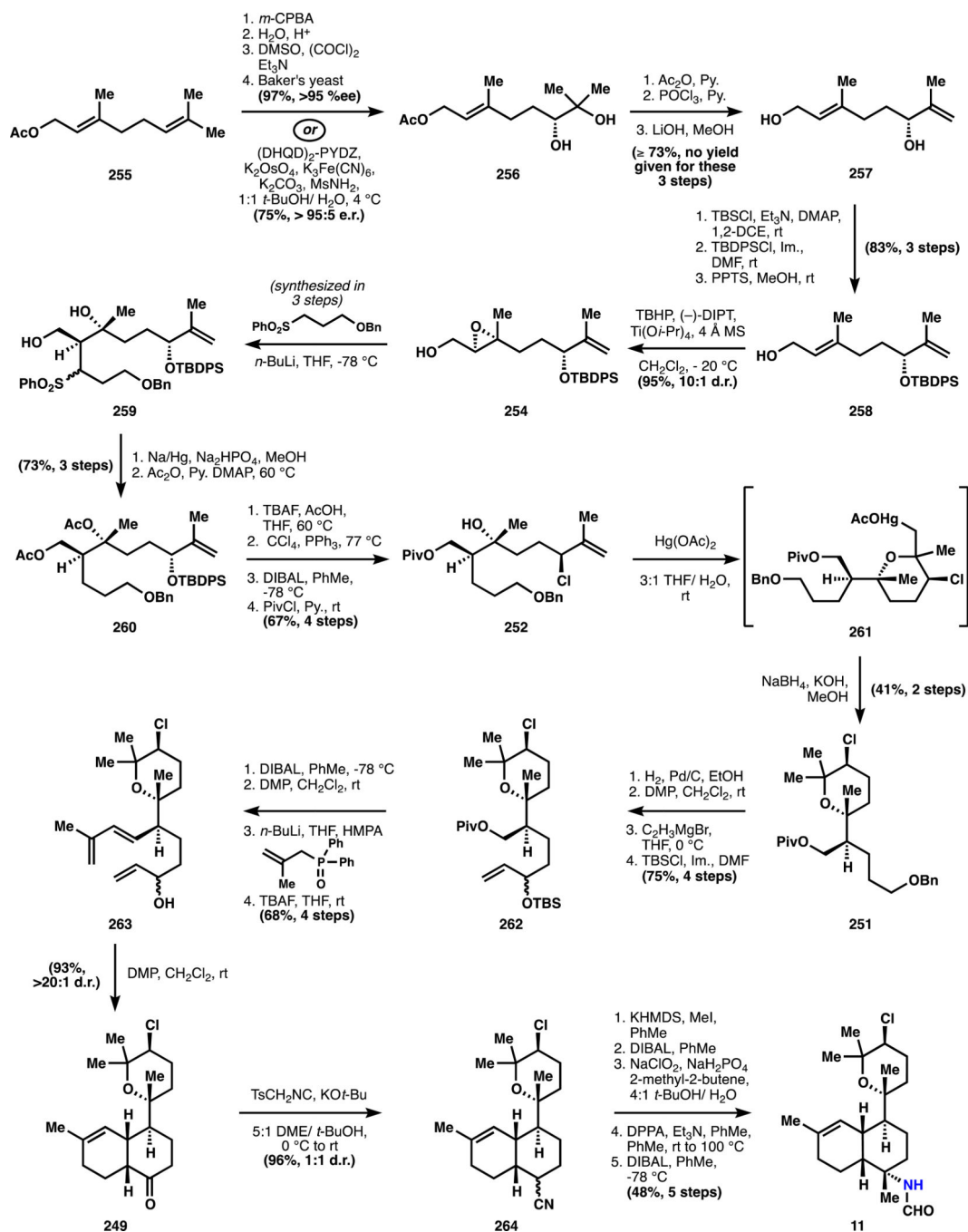
Figure 29.
Miyaoka's and Yamada's synthesis of **11**.
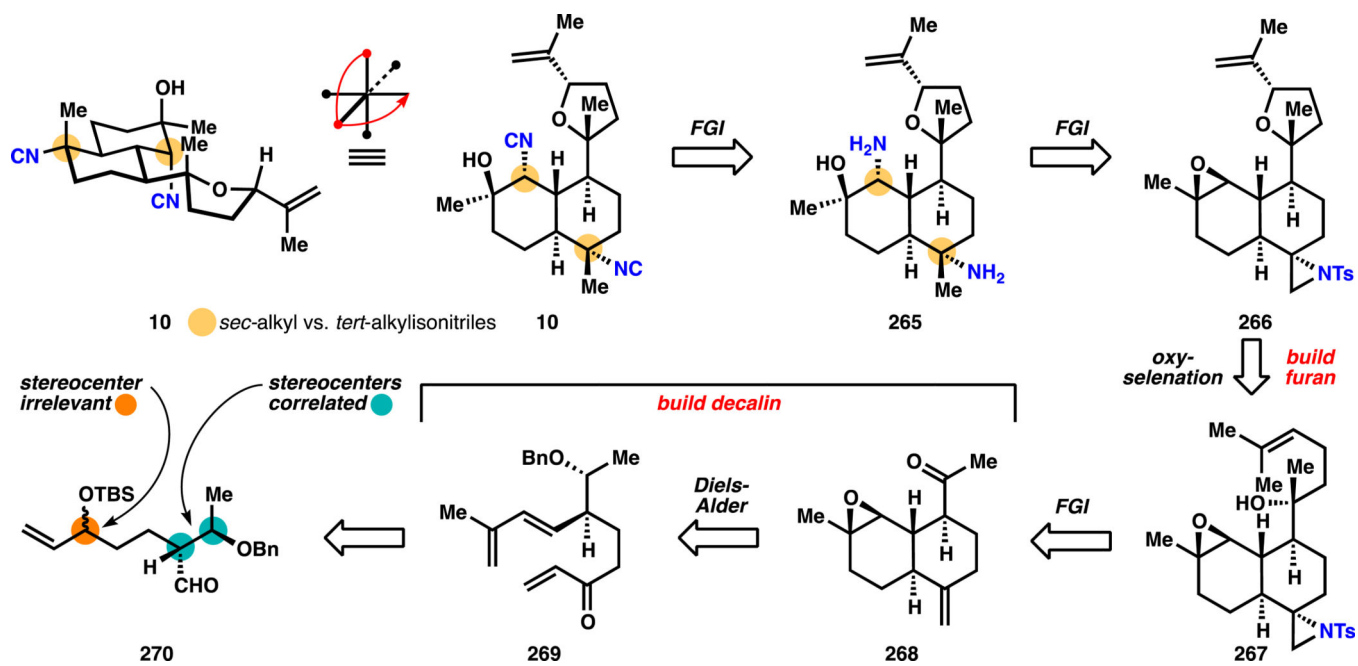
Figure 30.
Wood's analysis of **10** uses substrate control of most stereochemistry.
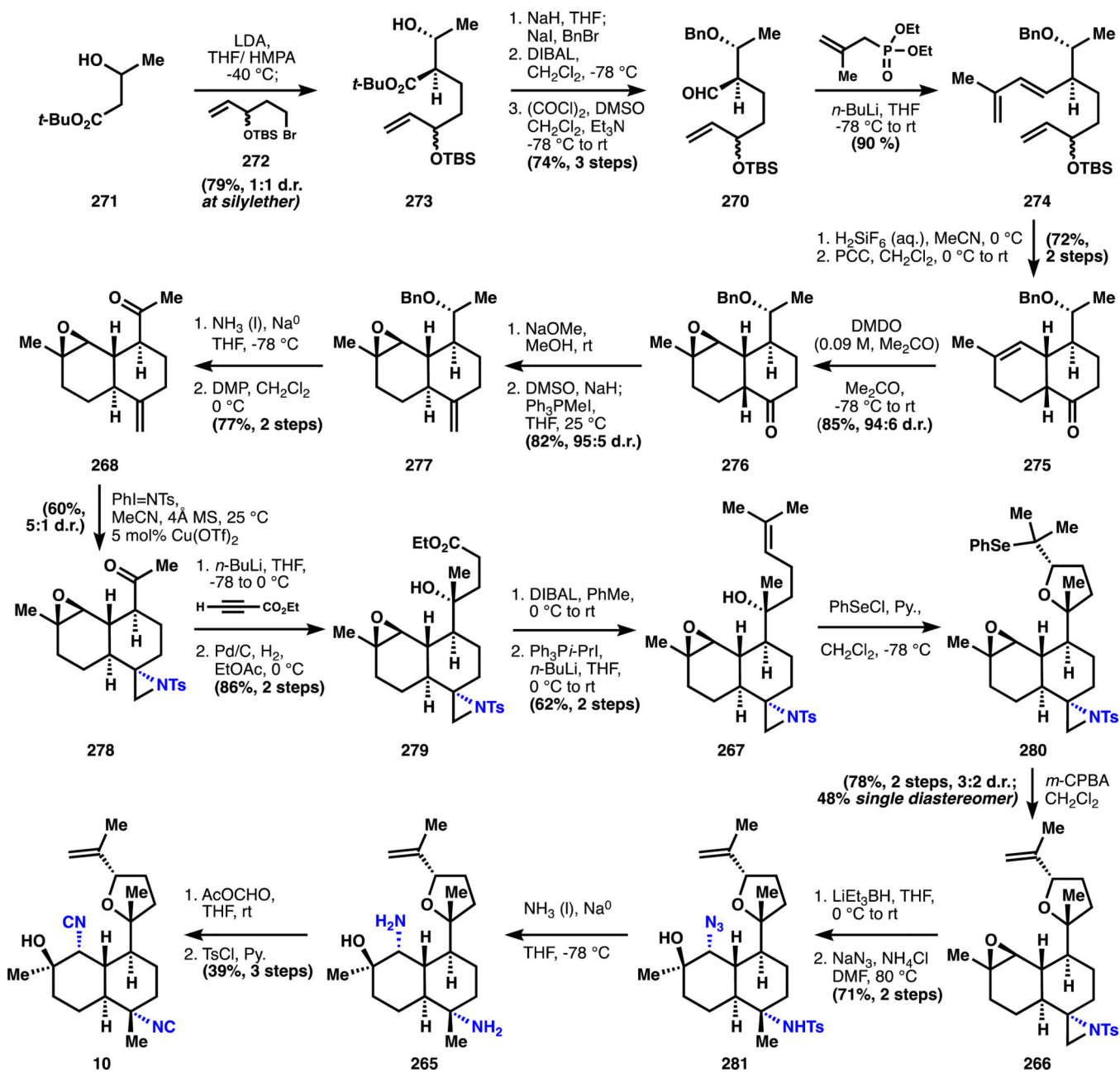
Figure 31.
Wood's synthesis of kalihinol C (**10**).
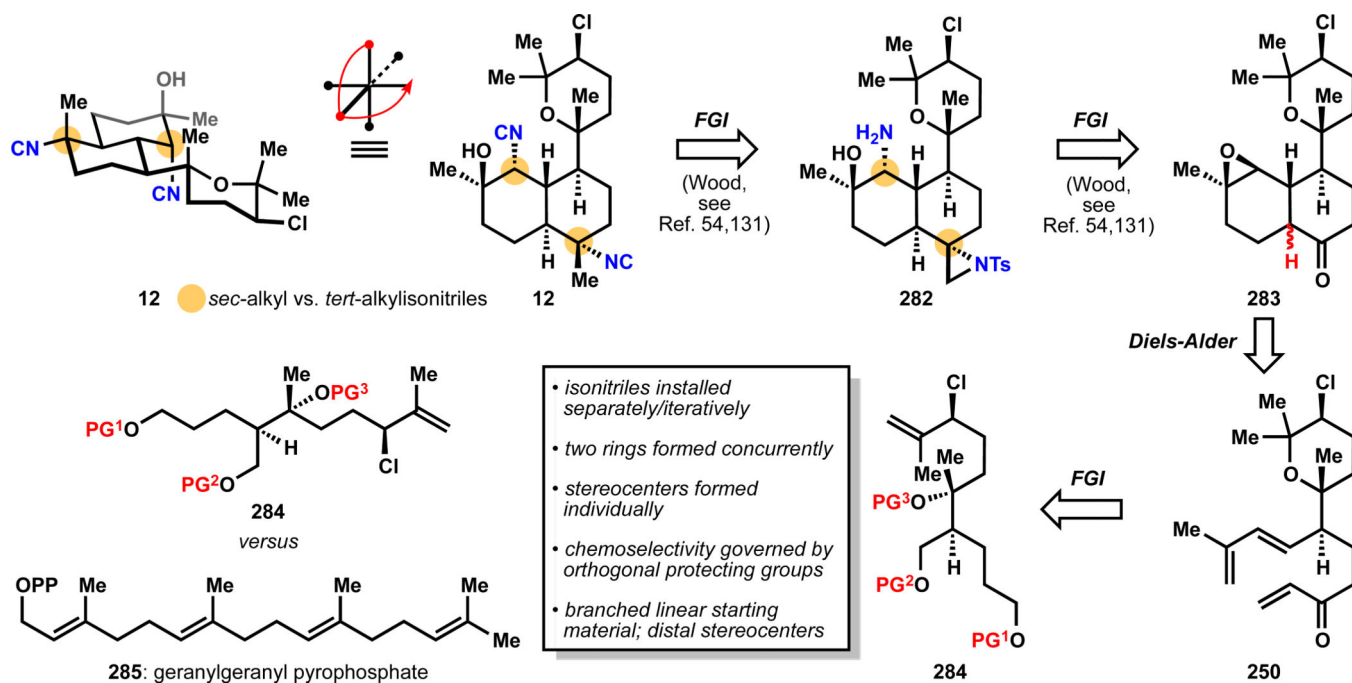
Figure 32.
Miyaoka's analysis of **12** relies on preinstalled stereocenters (e.g. **284**) prior to ring building, in contrast to the hypothesized biosynthesis of **12** from **285**.
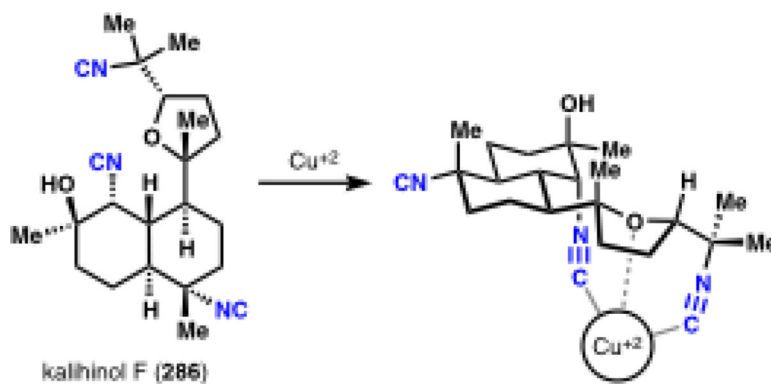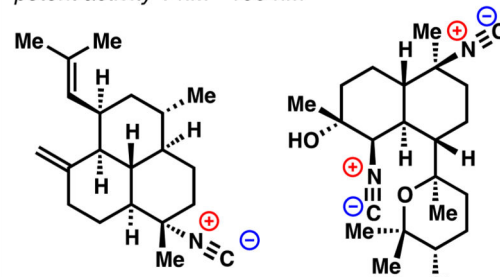
Figure 33.
Kalihinol F copper chelation model.
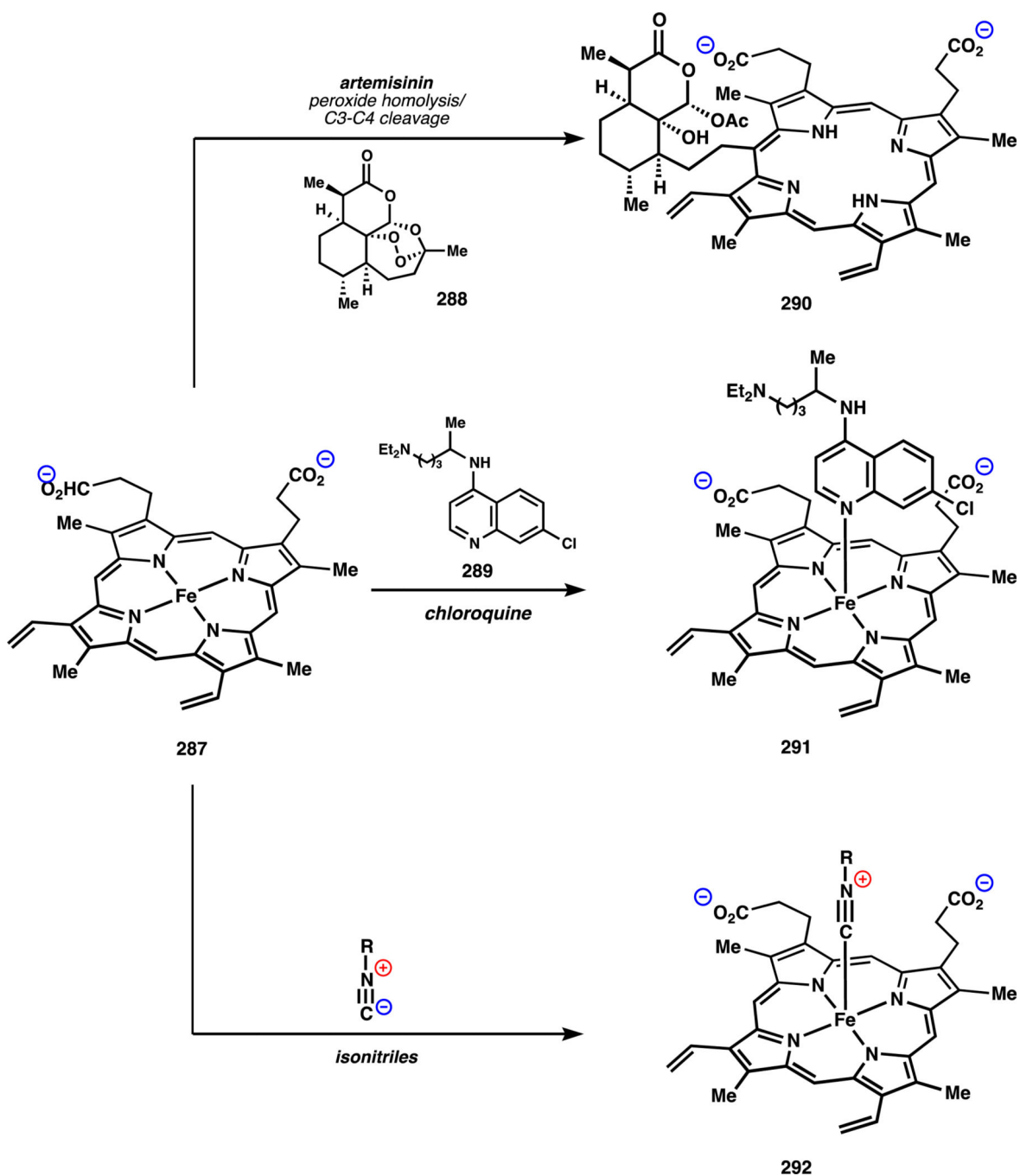
Figure 34.
Small molecule/heme interactions.
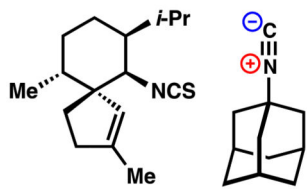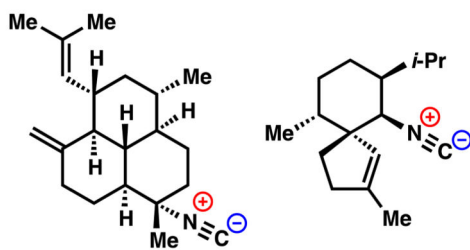
Figure 35.
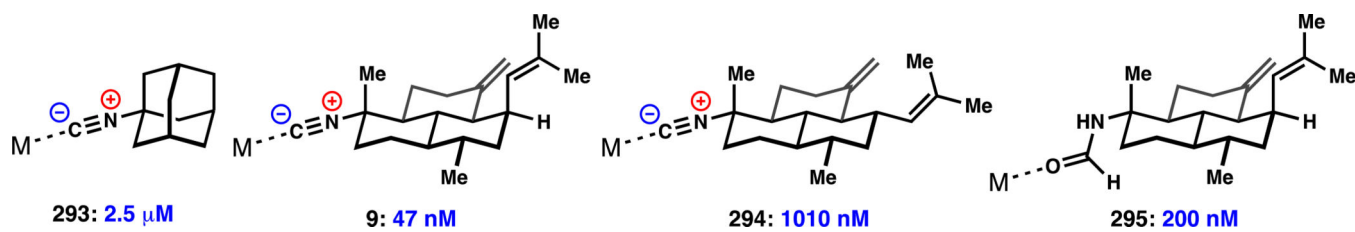Representative anti-malarial (*Plasmodium falciparum*) activity.[clv,clvii,clix,clx]

Figure 36.
Metal binding alone does not explain SAR.