Design and Synthesis of Molecular Scaffolds with Anti-infective Activity

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I. Introduction

Over the last five decades, not only have few novel classes of antibiotics been discovered, none of them have earned a central position in the antibiotic market. Indeed, the vast majority of newly-approved antibiotics are derivatives of those discovered more than 50 years ago.¹ An increasing number of pharmaceutical companies have exited from antibiotics research and, unsurprisingly, fewer antibiotics are approved each year.² Some have (erroneously) interpreted this trend as proof that there is a sufficient antibiotics library to fulfill medical need. However, even a cursory survey of newspaper headlines will show that the adaptability of microbes has begun to outstrip the advances of pharmaceutical research.

One need look no further than the emergence of antibiotic-resistant³ bacterial strains to highlight the need for new classes of antibiotics.^{1,2} From the appearance of several cases of life-threatening methicillin-resistant *Staphylococcus aureus* (MRSA) infection in 2008⁴ to the escalating incidence of tuberculosis (TB), an often deadly infection by *Mycobacterium tuberculosis* that claims a life every ten seconds,⁵ it is clear that antibiotic resistance is a serious problem for modern society. Further example is provided from 2011, when Germany was suffering from a *Escherichia coli* outbreak in which over 3,500 people were infected by a mutant strain within three months.^{6a,b} Although antibiotics are not considered an appropriate treatment for diarrhea caused by *E. coli* infection,^{6c,d} the unprecedented antibiotic-resistant strain found in this incident was a harbinger of more general resistance throughout the entire species. The resistance in this case, like many others, has been proposed to arise from inappropriate and excessive antibiotic use. As a result of this misuse, even vancomycin (**14**), often considered the last line of defense against serious bacterial infection, is now ineffective against several strains of resistant bacteria, including vancomycin-resistant *Staphylococcus aureus* (VRSA).⁷

The alarming decline in antibiotics research cannot be attributed to any single cause. It is tempting to blame the limited profitability of antibiotics due to the overwhelming majority of chronic bacterial infections occurring in developing countries which lack the economic power to purchase them.⁸ However, the fact that thorough, well-funded antibiotic discovery projects in industry have met with little success illustrates that the problem is also scientifically non-trivial.⁹ Regardless, it has been recognized that the battle with drug-resistant bacterial strains is a crucial challenge for human society and legislation is frequently discussed towards both better regulating the use of antibiotics and incentivizing antibiotic research.¹⁰ Currently, several non-profit associations (e.g., the *TB Alliance* and the *Gates Foundation*) and drug companies are backing initiatives to fight bacterial infections, including supplying discounted drugs to developing countries and continuing intensive research on infectious disease. Unfortunately, these efforts have proven insufficient thus far to stem the tide of antibiotic resistance, suggesting that more research in this area is necessary.

Most of the known antibiotics were isolated directly from living organisms or derived from natural products, but there are some classes of antibiotics that have been identified by screening synthetic small molecules.^{11,12} Currently, the market is dominated by only a few classes of traditional antibiotics (Figure 1A): β -lactams (including penicillins (**1**)¹³ and cephalosporins (**2**)¹⁴), quinolones (**3**),¹⁵ and macrolides (**4**);¹⁶ these major classes constitute about 75% of the market. The tetracyclines,¹⁷ aminoglycosides,¹⁸ and other classes of antibiotics (Figure 1C) constitute the remainder.¹⁹ Only a few novel classes of antibacterial compounds (e.g., pseudomonic acid,²⁰ oxazolidinones,²¹ lipopeptides,²² and platensimycins²³) have been identified in the past half century.²⁴

(c)

Figure 1. Chemical structures of traditional and underexplored antibiotic scaffolds. (a) Major antibiotic families in the market. (b) Antibiotic sales by class in 2009 in US\$ billions.¹⁹ (c) Selected naturally- and synthetically-derived antibiotics. Core scaffolds are in black and variable substitutions are in red; for compounds belonging to a particular class membership is indicated within parentheses.

Antibiotics in the same class usually suppress bacterial growth by inhibiting the same cellular target. When the targeted bacterial cellular component has changed due to genetic mutations, the interaction between a specific antibiotic and its target might be disturbed, resulting in resistance.²⁵ Resistance could also occur if bacteria evolve to have (1) a hampered diffusion or transport mechanism, (2) an alternative metabolic pathway no longer susceptible to the antibiotics, (3) improved detoxification system (*e.g.*, metabolic degradation and efflux pumps), or (4) any other alterations of pathways in which antibiotics are involved.³ In order to combat this resistance crisis there is an urgent need to find and develop new antibiotics with novel mechanisms of action. Not only are modern techniques (*e.g.* genomics-derived high-throughput screening) being developed for discovering new antibiotic scaffolds,²⁶ there are also discussions on sustainable ways to optimize efficiency and shelf life, such as combination therapies and a shift in R&D focus from broad-spectrum to narrow-spectrum antibiotics.²⁷

Scheme 1. Convergent assembly of structurally diverse 6-deoxytetracyclines by Myers.

Figure 2. Promising new scaffolds in antibacterial drug development.

Our group has a long-standing interest in developing novel synthetic strategies to construct the molecular scaffolds of secondary metabolites and other biologically active compounds. Historically, chemical synthesis can and has offered an alternative to reliance on the isolation of natural products from what are often scarce sources. Additionally, the flexibility of the former provides an opportunity to produce a wide variety of analogues that would not be otherwise available from natural resources or semisynthetic modifications on natural products. Our entry into this area has been greatly encouraged by successful discovery and development of novel tetracycline analogues by Myers and co-workers (Scheme 1).²⁸ The naturally occurring tetracycline compounds contain a hydroxyl group at the C-6 position; interestingly, semi-synthetic studies revealed that 6-deoxytetracyclines show better stability with comparable antibacterial activity. Myers and co-workers leveraged *de novo* synthetic design to rapidly access 6-deoxytetracyclines, and, with this purely chemical approach, greatly improved accessibility to promising, new tetracycline antibiotics. Their remarkable effort demonstrates the power of organic synthesis as a tool to expand the diversity of naturally occurring scaffolds and encourages the chemical community to explore diverse synthetic strategies for other antibiotic scaffolds.^{1,29} This perspective article summarizes our prior syntheses of the promising antibiotic scaffolds shown in Figure 2 and emphasizes our focus on diverse strategies for contending with the problems of forging topologically intricate and stereochemically complex ring systems.

Figure 3. The abyssomicin family of antibacterial, marine natural products.

II. First total synthesis of abyssomicin C featuring a nature-inspired, intramolecular Diels–Alder reaction

Abyssomicin C (32), a novel anti-infective natural product, was first isolated from the marine actinomycete *Verrucosispora maris* AB-18-032 by Süßmuth and co-workers in 2004 (Figure 3).³⁰ This compound contains a unique structural topology, featuring a tetronic acid moiety, an oxabicyclo[2.2.2]octane system, and an 11-membered macrocycle containing an α,β -unsaturated ketone motif. Abyssomicin C is considered an attractive lead structure in drug development for its unique mode of action: suppressing bacterial growth *via* inhibition of *para*-aminobenzoic acid (PABA) biosynthesis from chorismate, a pathway commonly found in many microorganisms but, importantly, not in humans.^{30,31} Interestingly, among the first three identified compounds within the abyssomicin family (abyssomicins B, C and D), only abyssomicin C showed antibacterial activity (MIC = 4 $\mu\text{g/mL}$ against MRSA; MIC = 13 $\mu\text{g/mL}$ against VRSA). Based on those data, a hypothesis was put forward that the oxabicyclo[2.2.2]octane system might serve as a mimetic of chorismate to the enzymes, whose nucleophilic side chains bind to the enone domain in abyssomicin C.³⁰ Aside from *Verrucosispora*, several compounds in the abyssomicin family have been found in other genera, such as abyssomicin I, which exhibits anti-tumor activity, from *Streptomyces*.^{32, 33}

Scheme 2. The concepts that guided our synthesis of abyssomicin C.

When considering the synthesis of natural products, biosynthetic pathways, shaped through untold years of evolution, exhibit exquisite creativity and efficiency.³⁴ Synthetic chemists, charged with making these compounds in the laboratory, have often been inspired to mimic and compete with Nature's designs. Since its discovery in the 1920s, the Diels–Alder reaction has been a powerful synthetic tool to build molecular complexity³⁵ and, unsurprisingly, this highly productive pericyclic reaction is speculated to be involved in some biosyntheses.³⁶

A consideration of the full structure of abyssomicin C led to a plan to construct the oxabicyclo[2.2.2]octane substructure from an epoxidation/ring opening sequence executed on the cyclohexene moiety of **44**, a tricycle that might emerge from a biomimetic intramolecular Diels–Alder reaction on trienone **43** (Scheme 2). Substrate **43** was envisioned to arise from three fragments *via* two, sequential carbonyl addition reactions.³⁷

Scheme 3. Total synthesis of abyssomicin C *via* a biomimetic intramolecular Diels–Alder reaction. DMDO = dimethyldioxirane; DMSO = dimethyl sulfoxide; *para*-TsOH = *para*-toluenesulfonic acid monohydrate.

Intermediate **43** proved to be a viable Diels–Alder substrate; however, its handling was found to be non-trivial (Scheme 3). In an effort to avoid isolating **43**, a one-pot β -elimination/Diels–Alder sequence on precursor **45** was planned. Through screening, it was found that treating **45** with lanthanum(III) triflate in toluene, with heating, promoted the desired cascade, yielding tricycle **44** as a single diastereomer. A site- and diastereoselective epoxidation of **44** followed by demethylation of the tetronic ester then furnished precursor **46** for the proposed final step. While the added stabilization through conjugation within the tetronic acid system depressed the nucleophilicity of the hydroxyl group in **46** under basic conditions, this hydroxyl will initiate a smooth trans-diaxial opening of the epoxide ring under acidic conditions to give a separable 1:1 mixture of the natural product abyssomicin C (**32**) and its related isomer **47** (Figure 4).³⁷

Figure 4. abyssomicin C and *atrop*-abyssomicin C.

Nicolaou and co-workers also encountered isomer **47** in the course of their chemical synthesis of abyssomicin C and were the first to confirm its structure by the method of X-ray crystallographic analysis; the isomer **47** that also arose when we treated compound **46** with acid is a stable atropisomer of abyssomicin C (**32**) (Figure 4).^{33f,g,h} The previously isolated abyssomicin C (**32**) and this new compound (**47**) differed in the orientation of the 11-membered ring moiety, a distinction made due to the rigidity of the skeleton.^{33f,g,h} Intrigued by this observation, Süßmuth and co-workers analyzed the culture filtrate from *Verrucosispora* and confirmed the presence of *atrop*-abyssomicin **47** in the strain,³⁸ finding that it is actually a major component in the naturally occurring extracts and that the compound readily undergoes isomerization to abyssomicin C (**32**) under the original, acidic isolation conditions.³⁸

The chemical synthesis of abyssomicin compounds has provided opportunities for further research on their biological and pharmacological properties. Not only did the biomimetic synthesis featuring the Diels–Alder reaction help elucidate the biosynthesis pathway *in vivo*,³⁹ it also provided additional abyssomicin-like compounds (such as stereoisomers and advanced intermediates) for biological evaluation.⁴⁰ Chemical modifications to the enone moiety have helped confirm its role as an electrophile for the covalent modification of enzymes, with Cys-236 of ADC synthase being validated as an active nucleophilic partner.⁴¹ This insight provides yet another example of the utility of chemical synthesis.

Figure 5. The guanacastepene family of fungal-derived natural products.

III. Construction of the tricyclic architecture of the guanacastepenes and related syntheses of guanacastepenes A and E

Clardy and co-workers first reported guanacastepene A (**29**) in 2000 after isolation from the fungus CR115. Subsequent to this, a family of diverse guanacastepenes was isolated from the same species (Figure 5), all of which share a core composed of fused 5-, 7-, and 6-membered rings.⁴² It has been found that guanacastepenes A and I, which contain a formyl group at C-4, display antibiotic activity through a cell membrane-disruption pathway. However, these compounds also exhibit toxicity to human red blood cells (RBCs) through an analogous mechanism, complicating their use as anti-microbials. Despite this problem, the potent activity exhibited against drug-resistant microbes is encouraging and efforts are ongoing to identify derivatives that can selectively lyse bacterial cells in the presence of human RBCs.⁴³

Scheme 4. Orbital overlap explanation for the ring-opening fragmentations of strained bicyclic ketones under one-electron reductant conditions. The C–C bond possessing maximal overlap with the activated carbonyl π -system fragments.

Our plan for synthesizing the guanacastepenes hinged on a concept that arose from early investigations of stereoelectronically controlled, reductive fragmentations of conjugated cyclopropyl and cyclobutyl ketones (Scheme 4). Indeed, it was known that

treatment of a strained carbon framework (e.g. **62**, **65**, and **67**) with a one-electron reducing reagent results in cleavage of the bond possessing the maximum overlap with the π -orbital of the activated carbonyl group.^{44,45} This fragmentation strategy was planned on multi-ring system **71**, in which a selective ring opening would directly generate the 5-7-6 tricyclic core structure of the guanacastepenes.⁴⁶ The original design called for a carbonyl addition of an organometallic reagent of the type **68** to aldehyde **69** to generate compound **70** (Scheme 5). An intramolecular [2+2] photocycloaddition would then afford **71**, which, when treated with a single-electron reductant, would form the putative radical anion **72**. The key, site-specific bond fragmentation (*vide supra*) would then reveal the central 7-membered ring and trigger a rapid β -elimination (see arrows in **73**) to complete the construction of the guanacastepene framework shown as **74**. For this attractive synthetic plan to work, the carbon-centered radical shown in **73** would need to undergo a simple reduction to the corresponding methylene shown in **74**.

Scheme 5. Original synthetic strategy towards guanacastepene core.

In the first test of this concept, **75** and **76** were elaborated to substrate **77** (Scheme 6) in a few steps. In the pivotal step, a reduction of pentacycle **77** with samarium iodide unexpectedly gave rise to the interesting tetracycle **78**.⁴⁶ Although unanticipated, this result was still encouraging. Seemingly, the originally designed fragmentation does occur on substrate **77**, but the putative carbanion **81** (or perhaps the secondary radical preceding **81**) readily undergoes an intramolecular, transannular conjugate addition to afford the cyclobutane-containing **78**.

Scheme 6. Original strategy led to an unexpected novel tetracycle.

Scheme 7. The revised convergent design towards guanacastepenes.

Scheme 8. Synthesis of the guanacastepene architecture featuring a [2+2]/reductive fragmentation/elimination sequence and its application in total synthesis of guanacastepenes A and E. DMSO = dimethyl sulfoxide; HMPA = hexamethylphosphoramide; *m*-CPBA = 3-chloroperoxybenzoic acid; PMP = *para*-methoxyphenyl; PPTS = pyridinium *para*-toluenesulfonate.

Modified substrate **82** (Scheme 7) was designed with the aim of solving this problem by circumventing the formation of the enone motif from the β -elimination, which had previously occurred on **80** (Scheme 6). Instead, an enolate trapping of **84** by a reagent like phenylselenenyl bromide (PhSeBr) would generate **85**, which would be available for a late-stage elimination. The implementation of this revised strategy commenced with a Stille coupling between fragments **86** and **87** (Scheme 8), followed by the [2+2]/reductive fragmentation/enolate trapping/elimination sequence from **88**. This sequence of chemical reactions permitted access to the advanced guanacastepene intermediate **90**. From this vantage point, guanacastepenes A and E were available in a few steps each,⁴⁶ providing a short, reliable synthesis to allow for later diversification.

IV. A synthesis of the reported structure of edaxadiene enabled a structure revision

After pathogenic microorganisms enter a macrophage cell and are absorbed by the phagosome, the phagosome will be induced to fuse with a lysosome, resulting in the foreign cell being digested in a low pH environment.⁴⁷ Interestingly (and alarmingly), certain pathogens, such as *Mycobacterium tuberculosis*, are able to inhibit this process. It is known that this inhibitive effect is partially related to mycobacterial cell surface lipids, but other effectors of this inhibition are still unclear.⁴⁸ In 2009, Peters and co-workers isolated a diterpene compound, edaxadiene (**33**), from wild-type *M. tuberculosis* (Figure 6)⁴⁹ demonstrating a close correlation with tuberculosis activity. Indeed, the genetic operon responsible for the *in vivo* production of edaxadiene was only observed in pathogenic strains and edaxadiene was shown *in vitro* to directly inhibit phagosome maturation.^{49,50} Although **33** itself is not an effective antibiotic, efforts to unveil the biosynthetic pathway of **33** and its molecular target in host cells are underway. Towards better understanding and combating this problematic characteristic of *M. tuberculosis*, increased access to edaxadiene and related compounds through chemical synthesis would be invaluable.

Figure 6. Original proposed structure of edaxadiene.

The originally proposed structure of **33** contains a halimane skeleton containing five contiguous stereogenic centers in its bicyclo[3.3.1]nonane framework with one quaternary stereocenter not assigned. In our approach to the problem of synthesizing this structure, an intramolecular ketone allylation was envisioned to convert enone **95** to the unusual bicyclo[3.3.1]nonane structure **96** (Scheme 9). A subsequent annulation of the left-most ring would then furnish **97** and give rise to the proposed structure of edaxadiene (**33**) after deoxygenation.⁵¹

Scheme 9. Synthetic strategy towards "edaxadiene".

The desired annulation on precursors **98a** and **98b**, respectively, could be effected *via* an oxidative cyclization in the presence of manganese(III) acetate and copper(II) acetate, giving bicyclo[3.3.1]nonanes **96a** and **96b**, respectively (Scheme 10).⁵² Even at this early stage, however, a discrepancy between these synthetic intermediates and previously reported ¹³C shifts at C-13 ($\Delta\delta > 40$ ppm) started to appear. To investigate this emerging NMR data difference, both **96a** and **96b** were converted into alkenes **100a** and **100b**, respectively. Although the volatility of these compounds limited their isolated yields, close relatives of the proposed edaxadiene structure (**33**) were available for comparison. Through ¹³C NMR analysis, a substantial mismatch for the quaternary carbons supported the hypothesis that the previously assigned edaxadiene structure might be incorrect. A survey of the literature revealed a related, known compound, nosyberkol (**101**), which was first isolated from the Red Sea sponge *Raspailia* sp.⁵³ and has been reported as a metabolite of *M. tuberculosis*.⁵⁴ However, the fact that the NMR spectra of nosyberkol and edaxadiene (**33**) were obtained in different deuterated solvents complicated the comparison of their spectral data.

Scheme 10. Discrepancies in ¹³C NMR chemical shifts indicated incorrect structural assignment of edaxadiene.

Scheme 11. Synthesized nosyberkol confirmed a structural revision of “edaxadiene”.

To interrogate whether the structure reported as **33** was in fact nosyberkol (**101**), a rapid synthesis of **101** was designed and executed (Scheme 11). The synthetic nosyberkol thus obtained was a mixture of inseparable diastereomers, the NMR data of which matched those reported for edaxadiene.⁵⁵ Furthermore, this result indicated that the previously isolated natural product was in fact a mixture of epimers at the allylic position. Finally, nosyberkol (**101**) was afforded by using the conditions reported to convert

tuberculosinol (**94**) to “edaxadiene (**33**)”, with further studies showing that *N,N'*-dicyclohexylcarbodiimide (DCC) is not required to trigger this transformation.

The structural revision of edaxadiene and concise synthesis of its actual structure, nosyberkol (**101**), exemplifies an important role of chemical synthesis: it not only serves as an alternative resource for supplying larger quantities of scarce natural products and related compounds for asking important biological questions, but also provides a means to support or refute tentatively proposed structural and configurational assignments of naturally occurring compounds.⁵⁶

Figure 7. Hirsutellones, pyrrocidines and GKK1032s share a common skeleton.

V. Rapid construction of hirsutellone cores featuring a dioxenone-opening/intramolecular Diels–Alder/intramolecular ketene-trapping cascade

Recently, a new class of polyketide antibiotics has been isolated from various fungi, a class including the hirsutellones from *Hirsutella nivea* BCC 2594 and *Trichoderma* sp. BCC 7579,⁵⁷ the GKK1032 compounds from *penicillia*,⁵⁸ and the pyrrocidines from *LL-Cyan426* (Figure 7).⁵⁹ All of these compounds share a common and hitherto unknown skeleton containing a decahydrofluorene subunit and a highly strained, oxygen-containing macrocycle. Although the specific mode of action for these compounds is unclear, their potent antimicrobial activity has attracted the attention of the antibiotic research community.^{57,58,59}

Hirsutellones A (**104**), B (**30**) and C (**107**) share the aforementioned decahydrofluorene moiety with identical patterns of substitution and stereochemistry, with their differences being focused on their macrocyclic subunits. Towards accessing these compounds and related analogues, a general synthetic strategy was proposed to build the hirsutellones, wherein precursor **113** could be constructed rapidly from four fragments *via* a pair of Wittig reactions⁶⁰ and a Tsuji–Trost etherification (Scheme 12).⁶¹ When heated, a retro-hetero-Diels–Alder reaction could occur on the dioxenone moiety of **113**, and the resulting intermediate **114** would undergo a highly productive intramolecular ketene addition/Diels–Alder cascade to furnish an advanced hirsutellone scaffold (**115**) suitable for further modification.⁶²

Scheme 12. Synthetic strategy towards hirsutellone B.

Compound **116**, approximately 15 linear steps from commercially available compounds, was prepared in order to demonstrate the viability of a simplified dioxenone-opening/intramolecular Diels–Alder sequence with methanol present as a co-solvent to trap the intermediate acyl-ketene (Scheme 13). The envisioned four-fragment strategy mentioned above proved amenable to the construction of a variety of advanced precursors, including **119**, **122** and **125**, and each of these smoothly underwent the proposed dioxenone-opening/intramolecular Diels–Alder/intramolecular ketene-trapping sequence in respectable yields.^{62,63}

Efforts to advance these and other, related intermediates in pursuit of a total synthesis of hirsutellone B using the strategy outlined above were ultimately unsuccessful.⁶³ Attempts to join C-17 and C-1' utilizing a multitude of methods to construct the γ -lactam (**115** to **30**, Scheme 12) failed to produce any of the desired bond formation, a result postulated to arise from a lack of proximity in such a rigid ring system. The variety of synthetic studies towards the hirsutellones and other natural products containing the decahydrofluorene core⁶⁴ include two total syntheses of hirsutellone B, reported by the Nicolaou^{65a} and Uchiro^{65b} groups, respectively. Critically, Nicolaou and coworkers constructed the highly strained 13-membered macrocycle by a Ramberg–Bäcklund reaction, while Uchiro used a copper-catalyzed Ullmann-type etherification; both of these syntheses access the 13-membered motif *via* ring-contraction from a less strained 14-membered macrocycle (or metallomacrocycle) and share a conceptual similarity with our original approach.⁶⁵ Having exhausted all other means for constructing the *para*-cyclophane motif, we found that we could modify our retro-hetero-Diels–Alder/intramolecular Diels–Alder sequence to intercept a late-stage diol intermediate from the synthesis reported by Nicolaou,^{65a} thus achieving a formal synthesis of hirsutellone B.

Scheme 13. A tandem acylketene formation/Diels-Alder/intramolecular ketene trapping sequence afforded hirsutellone cores.
DMB = 2,4-dimethoxybenzyl.

VI. Rapid synthesis of a pleuromutilin 5-8-6 tricyclic skeleton.

Pleuromutilin (**31**), a diterpene containing a unique 5-8-6 tricyclic skeleton, was first isolated from fungi by Kavanagh in the early 1950s (Figure 8).⁶⁶ Pleuromutilin and its derivatives bind to the peptidyl transferase center of bacterial ribosomes, which are intrinsically different from human ribosomes, and hence selectively inhibit bacterial protein syntheses.⁶⁷ Naturally occurring pleuromutilin displays moderate activity against Gram-positive bacterial strains, and it was demonstrated that this activity could be enhanced by semi-synthetic modifications to the natural product, especially the C-14 glycolate ester.⁶⁸ Currently, there are three pleuromutilin antibiotics on the market: retapamulin (**128**), indicated for skin infection (the first pleuromutilin derivative approved for human use), tiamulin (**129**), and valnemulin (**130**), two veterinary drugs.⁶⁹ Unfortunately, the development of pleuromutilin and its relatives has been limited by their metabolic instability, with the C-2 and C-8 methylenes being rapidly hydroxylated *in vivo* by cytochrome P450 enzymes, particularly CYP3A4.⁶⁹

X-ray analysis (Figure 9) of the complexes between bacterial ribosomes and active pleuromutilin compounds has revealed their mode of binding, with key interactions arising from (1) the hydrogen bonds from the C-3 ketone of the cyclopentanone moiety, the carbonyl of the C-14 glycolate or thioglycolate ester, and the C-11 hydroxyl group on the 8-membered ring; and (2) van der Waals contact between the alicyclic portions of the pleuromutilins and hydrophobic portions of the ribosomes.⁷⁰ Using this insight,

we envisioned that chemical synthesis could be leveraged to produce unnatural pleuromutilin analogues that might be stable to metabolism while retaining or enhancing potency and maintaining its exquisite selectivity for the bacterial ribosome.

Figure 8. Pleuromutilin and its derivatives.

Figure 9. Structure of tiamulin within the peptidyl transferase center (PTC) (Copyright 2004 Blackwell Publishing Ltd).^{70a}

Figure 10. Targeted pleuromutilin-like scaffold.

Scheme 14. Synthetic strategies towards targeted pleuromutilin derivatives.

The pleuromutilin scaffold is readily available from biosynthetic fermentation⁷¹ and has traditionally served as a practical starting point for many successful semi-synthesis efforts, complementing the fully synthetic approaches explored

contemporaneously by several groups.⁷² We recognized the extreme difficulty semi-synthetic approaches faced to chemically modify certain portions of the pleuromutilin skeleton, particularly the metabolically labile C-8 methylene and, taking inspiration from the binding mode observed by X-ray analysis,⁷⁰ we were inspired by the potential activity of simplified scaffolds like **131** (Figure 10) compared to the more heavily-substituted natural pleuromutilin **31**. The proposed synthetic scaffold would maintain all the key elements for hydrogen bonding, previously proven necessary for activity, but the absence of most other functionality was proposed to add flexibility to the synthetic design process and, hopefully, lessen metabolic instability. The planned synthetic strategy called for a diastereoselective conjugate addition of cyanide to enone **132**, giving *cis*-hydrindanone intermediate **133** (Scheme 14). Addition of an alkenyl anion equivalent to the aldehyde would furnish allylic alcohol **134**. The aldehyde derived from selective reduction of the nitrile would then be subjected to an intramolecular allyl addition to yield **131**.⁷³

As expected, **137** was available from a diastereoselective cyanide addition to **136** followed by acetal formation with ethylene glycol (Scheme 15). After ozonolysis of the terminal olefin, the resulting aldehyde was coupled with related vinyl halides **138** *via* Nozaki–Hiyama–Kishi reaction, giving rise to separable epimeric alcohols **139a** and **139b**. Epimers **139a** and **139b** were advanced to epimeric products **140a** and **140b**, which, upon treatment with chromium(II) chloride, could be converted to the diastereomeric **141a-d**, each containing the desired 8-membered ring. Interestingly, substrate **140a** participates in this ring closure much more rapidly than its epimer, **140b**. Indeed, **140a** smoothly underwent the chromium-mediated ring formation regardless of the protecting group on the C-11 oxygen, while **140b** was much more sensitive to the steric environment at this position, further emphasizing the large effect of this subtle difference. This approach was used for the successful construction of several novel pleuromutilin-like scaffolds.⁷³

Scheme 15. New pleuromutilin scaffolds are available *via de novo* chemical synthesis. *para*-TsOH = *para*-toluenesulfonic acid monohydrate; TMS = trimethylsilyl; TES = triethylsilyl; DIBAL-H = diisobutylaluminum hydride; DMF = *N,N*-dimethylformamide.

Scheme 16. A representative pleuromutilin-like compound (X-ray crystallographic data for compounds **142b** have been deposited at the Cambridge Crystallographic Data Centre under the number CCDC 861251). DCC = *N,N'*-dicyclohexylcarbodiimide; DMAP = 4-dimethylaminopyridine.

Rapid diversification of these intermediates was achieved through the incorporation of various side-chains at the C-14 position, with a global deprotection affording the desired pleuromutilin analogues, such as **144b** (Scheme 16). It was found that removal of the hydrophobic substituents on the pleuromutilin-like compounds greatly improved their metabolic stability to cytochrome P450 enzymes but came at the cost of decreased antimicrobial activity, typified by the activities of both **144b** and its C-14 epimer which were found to be significantly lower than tiamulin (**129**, Figure 8) against several panels of Gram-positive and Gram-negative bacteria.⁷⁴ Some of these synthetic pleuromutilin-like compounds did demonstrate moderate activity against an attenuated strain of *Mtb mc*²-7000, however, it was later found that these same compounds are inactive against a pathogenic tuberculosis strain, in laboratories affiliated with the *TB Alliance*. Unfortunately, it was also found that reducing the alkene substituent at C-12 to a sp³ carbon (in an effort to better model the naturally occurring substitution present in the pleuromutilin carbon framework) resulted in no increased antibacterial activity. Apparently, there exists a tradeoff between suppressing metabolic susceptibility and maintaining antibacterial activity, a postulated balance tied to the presence of hydrophobic groups on the pleuromutilin scaffold, but whose exact mechanism is currently unknown. We hope to apply these lessons towards the production of new, *de novo* synthetic scaffolds to access potent and metabolically stable pleuromutilin analogues.⁷⁵

Figure 11. Representative nitroimidazole antibiotics.

VII. Improved synthesis of PA-824

Nitroimidazole compounds have a long history of use as effective antibiotics (Figure 11).⁷⁶ Such compounds are prodrugs, only toxic once activated by bacterial redox systems *in vivo*, leading to fatal DNA damage through the action of unstable radical intermediates. Their success can be understood to arise from two main factors: a constant influx of the compound due to the concentration imbalance resulting from constant reduction of nitroimidazoles within the cell membrane^{77a} and excellent selectivity for bacteria (and other microorganisms) as the highly-reducing Fe^{II} ferredoxin required for their reduction is not present in the mammalian redox system.^{77b} Finally, in contrast to several other traditional antibacterial classes whose levels of efficacy are only useful for bacteria with high replication rates, nitroimidazole compounds show promising complementary activity against dormant or slow-growing organisms, such as *M. tuberculosis*.⁷⁸

PA-824 (**34**), a synthetic, bicyclic nitroimidazole anti-tuberculosis compound developed under the generic name pretomanid by *TB Alliance*, was discovered in 2000.⁷⁹ Previous synthetic approaches to PA-824 (**34**) started with explosive dinitroimidazole **149** and expensive epoxide **150**, and a synthetic sequence requiring a throughput-limiting four further chromatographic separations (Scheme 17).⁸⁰ To reduce the cost and increase the availability of PA-824 (**34**), as well as circumvent the safety issues surrounding dinitroimidazole **149**, we realized that a new approach would be needed. To avoid the costly and dangerous intermediates utilized previously, the new approach would construct the 6-membered oxazine ring from the non-explosive nitroimidazole scaffold **152** and a functionalized glycidol derivative **153**.⁸¹

Scheme 17. Synthetic strategies towards PA-824. TBS = *tert*-butyldimethylsilyl.

Scheme 18. Improved synthetic route for production of PA-824. TfOH = trifluoromethanesulfonic acid; DMF = *N,N*-dimethylformamide.

Importantly, synthesis of pseudosymmetrical glycidol derivative **153** starts from readily available diol **154** (Scheme 18). After acylation of the primary alcohol of **154** and benzylation of the secondary alcohol, the resulting alkyl chloride **158** can be coupled with commercially available, non-explosive nitroimidazole **159**, giving rise to **160**. Treatment of **160** with potassium hydroxide in methanol triggers a saponification/anionic cyclization/elimination sequence to furnish PA-824 (**34**) in good yield, on gram-scale, and with retention of the absolute configuration. The whole process from **154** to **34** only required two (opposed to the previously required four) chromatographic separations and two recrystallizations. This improved approach simplifies the synthetic process and provides a means for the large-scale production of PA-824 (**34**) for further studies,⁸¹ showing yet another instance where chemical synthesis can have a large impact in the pursuit of anti-microbials.

VIII. Conclusion

In this article, we revisited the chemical concepts underlying our syntheses of the promising antibacterial natural products abyssomicin C and guanacastepene E, our reassignment of the structure of the *M. tuberculosis* metabolite edaxadiene, our construction of several hirsutellone-like compounds, our effort to expand the depth and diversity of the pleuromutilin class of antibiotics by *de novo* chemical synthesis, and our short, scalable synthesis of the promising new antitubercular agent PA-824. While future research is needed to establish the therapeutic potentials of these particular classes of anti-infective agents, the ideas and methods of organic chemical synthesis will unquestionably be central to new efforts to combat the global threat posed by pathogenic bacteria through the development of new chemotypes for antibiotic development efforts.

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guanacastepene A (29)

hirsutellone B (30)

pleuromutilin (31)

abyssomicin C (32)

"edaxadiene" (33)
(originally reported strucutre)

PA-824 (34)