CHAPTER 1

Late-Stage Diversification of Natural Product Scaffolds:

A Tool for Synthetic and Biological Studies

1.1 INTRODUCTION

The following chapter is intended to present an overview of complex molecule diversification, including the motivations for conducting these studies, the various strategies developed for this purpose, and highlights of published reports. Considering the vast breadth of study in this open-ended and active research area, the present discussion will focus on strategies that involve late-stage diversification of natural product-inspired scaffolds. References for reviews and examples of studies using alternative strategies will be provided as appropriate.

1.2 OVERVIEW OF COMPLEX MOLECULE DIVERSIFICATION

Fine-tuned over thousands of centuries for specific biological roles,[1] natural products served therapeutic purposes from the dawn of the most rudimentary medical practices in

human civilization and continue to inspire drug development in today's highly technical world.[2] Tremendous advances in synthetic chemistry and biology research over the past half-century have greatly enhanced understanding of many biological processes for which natural products were evolved. The de-mystification of many natural products' roles in biology has enabled the performance of detailed studies correlating molecular structure with biological function, thereby providing the scientific community with opportunities to plan research strategies around the conclusions drawn from these investigations.[3] In line with this phenomenon, the past few decades have witnessed a surge in research programs aiming to derivatize complex molecules with the ultimate goal of discovering novel therapeutics and the concomittant aim of establishing powerful methodologies to facilitate complex molecule synthesis. Overall, the synthetic and medicinal insights gleaned from this type of research originate from a unique perspective complementary to those of pure total synthesis and methods development programs.

1.2.1 MOTIVATIONS

Central to any research program is the impact of the findings on the scientific community and beyond. The goals of complex molecule diversification programs are multi-faceted but center largely around studying the biological activities of non-natural structurally intricate compounds and preparing large quantities of the complex precursors to the aforementioned non-natural compounds. While the primary aim of using organic synthesis to study biology is of great significance to medicinal chemistry and drug development, the seemingly peripheral goal of executing multi-step synthesis of complex molecules should not be underestimated in its potential for generating impactful

information of high relevance to the chemical community. Together, the biological and synthetic implications derived from these investigations are what motivate scientists to devote significant effort to the diversification of complex molecular scaffolds.

1.2.1.1 BIOLOGICAL CONSIDERATIONS

Natural products have served therapeutic purposes for many centuries, and today most FDA-approved drugs available are small molecules, many of which are based on natural products.[2,4] Given the intimate relationship between complex molecules and drug development, a central theme of most research efforts in complex molecule diversification entails the biological evaluation of the derivative compounds generated. The specific disease area investigated can either be targeted based on knowledge of the biological activities of related known compounds (as is the case with natural product-based strategies) or left open to as wide a range as possible (as is the case with classic diversity-oriented synthesis approaches). In all cases, developing an understanding of the three-dimensional configurations of the complex molecule derivatives, especially in the context of interaction with the biological agent to be studied, is of paramount importance if meaningful conclusions about biological activity are to be made. Under the appropriate circumstances, unexpected observations in biological investigations could lead to significant discoveries about the mechanisms of activity among complex molecules and contribute to the potential for a given compound to form the basis of a drug development program.

1.2.1.2 SYNTHETIC CONSIDERATIONS

As a more immediate consideration, diversification studies also provide a foundation from which to develop an efficient and reliable synthetic route for accessing a complex molecular scaffold in large quantities. Unless the compound to be diversified is commercially available or accessible through semi-synthesis, a highly effective multi-step synthesis is generally required for the overall research program to succeed. While this consideration may resemble those of a traditional total synthesis project, the amount of late-stage material required for a successful diversification project generally exceeds what is necessary to complete a total synthesis since the number of potential targets is essentially limitless.[5] As such, constant optimization of the synthetic route to the main scaffold is common in diversification programs and often leads to the development of new methodologies or strategies to expedite the synthesis.

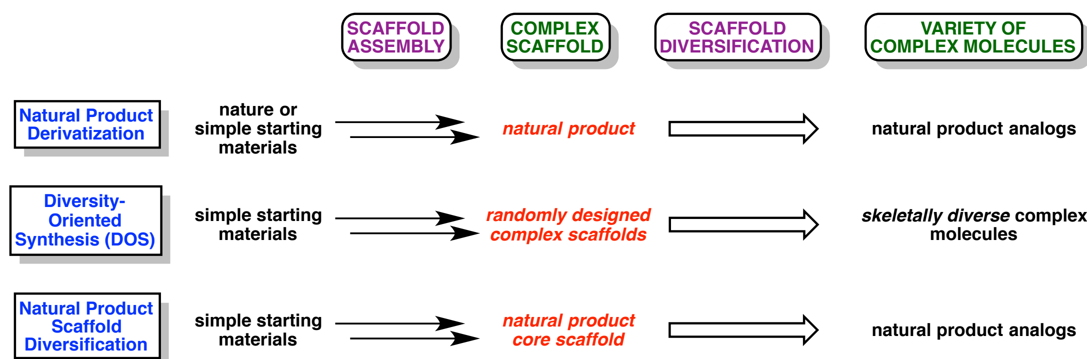
Once the core scaffold has been obtained in sizeable quantities, diversification studies also provide a viewpoint from which to examine the reactivities of complex frameworks. Unexpected outcomes of traditionally straightforward reactions often form the basis of efforts to adapt pre-established methodologies for the transformations of complex molecules, contributions which are likely to find use in many other synthetic endeavors. Another important synthetic consideration in the later stages of diversification projects is the characterization of all the non-natural compounds synthesized. Since accurate knowledge of molecular structure is vital to the validity of the structure-activity relationship (SAR) studies that form the backbone of biological assessment, significant effort should be expended on elucidating the intricate, unknown structures of the complex derivatives prepared. As no reference data exists for these non-natural compounds,

structure elucidation is often achieved through multi-dimensional NMR spectroscopy, X-ray crystallography, and high-resolution mass spectrometry, among other means.

1.2.2 STRATEGIES

Many approaches toward the diversification of complex molecules have been documented over the years. While each account bears unique nuances that evade classification, it can be useful to demarcate the myriad examples into three distinct categories: 1) natural product derivatization, 2) diversity-oriented synthesis, and 3) natural product scaffold diversification (Figure 1.1). Although all three types share similar attributes, the key differentiating factor is the nature of the scaffold to be diversified. This, along with subtle discrepancies in the motivations and philosophies, serves to delineate these strategies and highlight the major contributions of each approach.

Figure 1.1 Overview of strategies for complex molecule library preparation



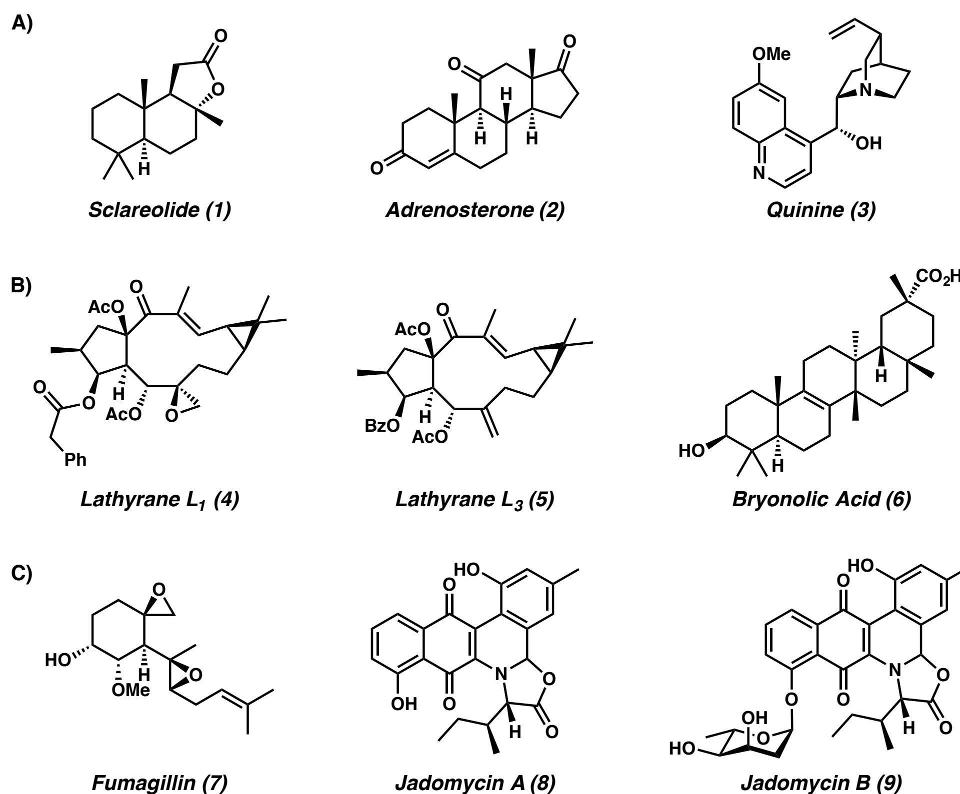
1.2.2.1 NATURAL PRODUCT DERIVATIZATION

Natural products are central to modern drug discovery efforts, as evidenced by the success of pharmaceuticals such as paclitaxel (anticancer), artemisinin (antimalarial), daptomycin (antibacterial), and morphine (analgesic).[6] While natural products and their derivatives have comprised many small molecule drugs since the 1940s,[7] interest in developing natural products as therapeutics began to wane in the 1990s due to challenges in identifying new biologically potent natural agents.[8] Furthermore, advances in synthetic methods enabling the rapid assembly of diverse molecular architectures encouraged the transition away from reliance on natural products and toward synthetic scaffolds.[9] However, extensive research over the last few decades has revealed that the considerable structural differences between typical synthetic scaffolds and natural products correspond to substantial disparities in biological activity.[10] Specifically, the differences in ring system complexity, percentage of sp^3-hybridized carbons, heteroatom content, and number of stereocenters contributed to significant structural variations that resulted in the synthetic scaffolds and natural products targeting different macromolecular receptors.[11]

Given the potential to complement the therapeutic benefits of synthetically derived lead molecules, enthusiasm for natural product research has been rejuvenated over the past decade. Armed with modern synthetic methods and techniques for conducting detailed SAR studies, chemists are well situated to build on the foundation established by previous natural product and synthetic scaffold research. Current research programs to create natural product analogs target compounds that incorporate the structural

complexity and physicochemical properties of natural products while employing efficient routes that enable rapid construction of the scaffold.

Figure 1.2 Starting points for derivatization studies: selected natural products available through A) commercial suppliers, B) extraction, or C) semi-synthesis



Considering the therapeutic effects exhibited by many natural products, one reasonable strategy for generating libraries of compounds for medicinal evaluation involves the direct modification of natural products themselves. Many are available from commercial suppliers, facilitating their use as starting points for library assembly.[12] For instance, numerous diversification studies have been carried out on the commercially available natural products sclareolide (**1**),[13] adrenosterone (**2**),[14] and quinine (**3**),[14,15] generating an abundance of derivatives in large enough quantities for extensive biological

evaluation (Figure 1.2A). Natural products obtained through extraction have also served as fine starting points for diversification studies, such as those conducted on the lathyrane diterpenoids L_1 (**4**) and L_3 (**5**)[16] and bryonolic acid (**6**),[17] among others (Figure 1.2B). Additionally, Furlan and co-workers demonstrated that extracts containing mixtures of several natural products could also be conveniently transformed into useful diversified analogs that could be screened for biological activity, further encouraging the use of natural products as library progenitors.[18]

Synthetically, natural products can be accessed through total synthesis or semi-synthesis, which entails enzymatic generation of the desired natural product. While diversification studies based on natural products arising from total synthesis have been accomplished, the high step counts of many total syntheses hinder the applicability of this strategy for accessing natural products as diversification scaffolds. In contrast, semi-synthesis has emerged as a useful approach toward this end, permitting facile production of compounds such as fumagillin (**7**)[19] and jadomycins A (**8**) and B (**9**)[20] to be used as diversification scaffolds (Figure 1.2C).

1.2.2.2 DIVERSITY-ORIENTED SYNTHESIS

Aiming to discover small molecules with therapeutic properties orthogonal to those of both natural products and pharmaceutical proprietary compounds,[21] diversity-oriented synthesis (DOS) is a relatively new research area, rising to prominence only within the past 15 years. DOS has been defined as "the deliberate, simultaneous, and efficient synthesis of more than one target compound in a diversity-driven approach."[22] The central principles of DOS assert that traditionally undruggable disease-related targets like

protein-protein interactions (PPIs) and protein-DNA interactions may be conquered by the ideally crafted small molecule therapeutic which differs in just the right aspects from currently available pharmaceuticals.[23] Since structure and function are generally related in small molecule therapeutics, DOS programs seek to vary as many aspects of compound libraries as possible, including scaffold structures, stereochemistry, and scaffold substituents.[19a] In effect, the DOS approach is opposite to that of the natural product derivatization strategy. Rather than seeking to uncover a derivative with enhanced potency toward a particular disease agent as natural product derivatization programs often do, DOS programs aim to study as many potential targets for therapeutic intervention as possible with the goal of elucidating their amenabilities to small molecule modulation. In accordance with this philosophy, DOS strategies seek to derivatize a wide range of molecular scaffolds rather than just one.

The ultimate goal of DOS is to explore the entirety of bioactive chemical space using functionally diverse small molecules. While this aim remains largely utopian in nature due to the astronomically high number of compounds this would encompass (about 10^{63} compounds of mass < 500 Da),[24] the recent adaptation of solid-phase synthetic methods to organic synthesis has made the rapid assembly of thousands of complex molecules a reality. Originally developed for polypeptide synthesis in the 1960s,[25] solid-phase techniques have simplified the purification processes for organic compounds,[26] enabling hundreds of reactions to be carried out in parallel, a logistical impossibility using traditional purification methods (e.g. silica gel column chromatography). While an exhaustive review of the myriad examples of DOS is outside the scope of this discussion,

there are numerous documented accounts[27] in addition to reviews[28] summarizing the successes and challenges of this growing area of research.

1.2.2.3 NATURAL PRODUCT-INSPIRED SCAFFOLDS/LIBRARIES

The final strategy for complex molecule library preparation to be discussed entails the modification of natural product-inspired scaffolds, often available as intermediates in a synthetic route to the natural product or independently designed to mimic the structure of a biologically potent natural product. Described by Danishefsky as "diverted total synthesis,"[5] this tactic incorporates advantageous qualities of both the natural product derivatization and DOS approaches to complex molecule diversification. Namely, the natural product-inspired scaffold can be strategically selected or designed to include a more diverse set of functional handles (reminiscent of DOS strategy) while still retaining the core structure of a biologically active natural product (similar to natural product derivatization). Furthermore, as an intermediate to the natural product, the chosen scaffold is more easily accessible through synthesis than the natural product in quantities appropriate for biological study. In this way, compounds generated through diversification of natural product-*based* scaffolds (not the natural products themselves) provide avenues for studying the biological activities of natural product families that may be challenging to access through total synthesis (for instance due to low-yielding endgame transformations).

Along the same lines, the natural product-inspired approach allows for the examination of natural product family hybrids as potential therapeutics. It is often the case that two or more natural product families share core structures but exhibit varying

biological activities. As such, combining salient features (e.g. oxidation states, substitution patterns, functional groups) of both families on the common carbon skeleton creates "hybrid" molecules that may exhibit heightened potency or even novel activity. Specific examples of the natural product-inspired scaffold diversification are outlined in the following section.

1.3 PREVIOUS DIVERSIFICATION STUDIES

True to the open-ended nature of this research area, there exists an abundance of literature detailing the diversification of complex molecules, and an exhaustive review of these studies would be highly impractical. Instead, the present discussion will focus on accounts that employ the natural product-inspired scaffold diversification strategy since this approach is the most relevant to the research described in the later chapters of this text. The following sections present highlights from studies based on one of three approaches: 1) diversification of a late-stage intermediate in a natural product total synthesis, 2) diversification of a scaffold independently designed to mimic a natural product core, or 3) diversification of a scaffold to access hybrid molecules between two or more natural products.

1.3.1 SCAFFOLD AS AN INTERMEDIATE IN TOTAL SYNTHESIS

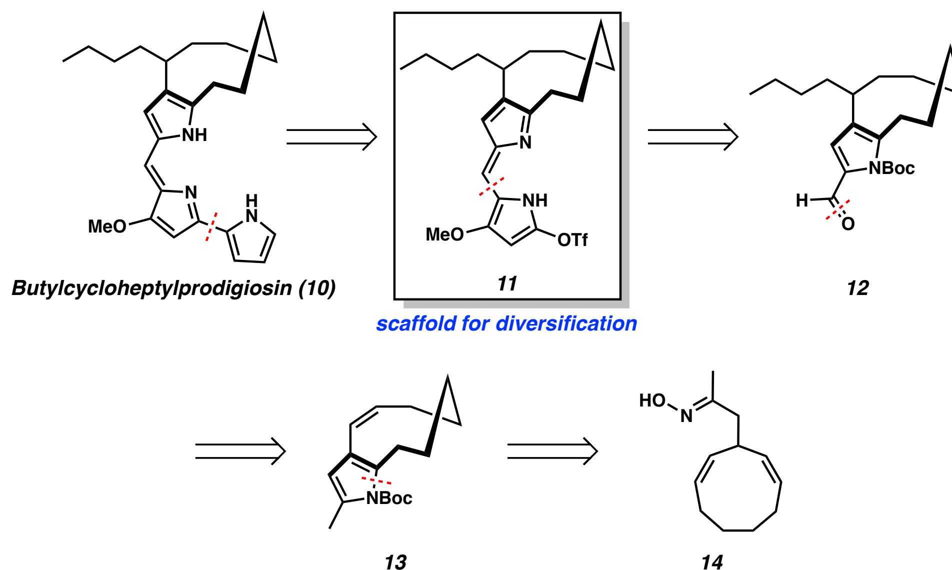
Diversification studies often originate seamlessly from natural product total synthesis research programs due to the ready availability of complex late-stage intermediates. Furthermore, total synthesis and diversification projects enjoy a symbiotic relationship in

that diversification of a late-stage intermediate for biological screening purposes also provides insights into core reactivity that may prove critical to the eventual success of the total synthesis.

1.3.1.1 FÜRSTNER'S BUTYLCYCLOHEPTYLPRODIGIOSIN SYNTHESIS

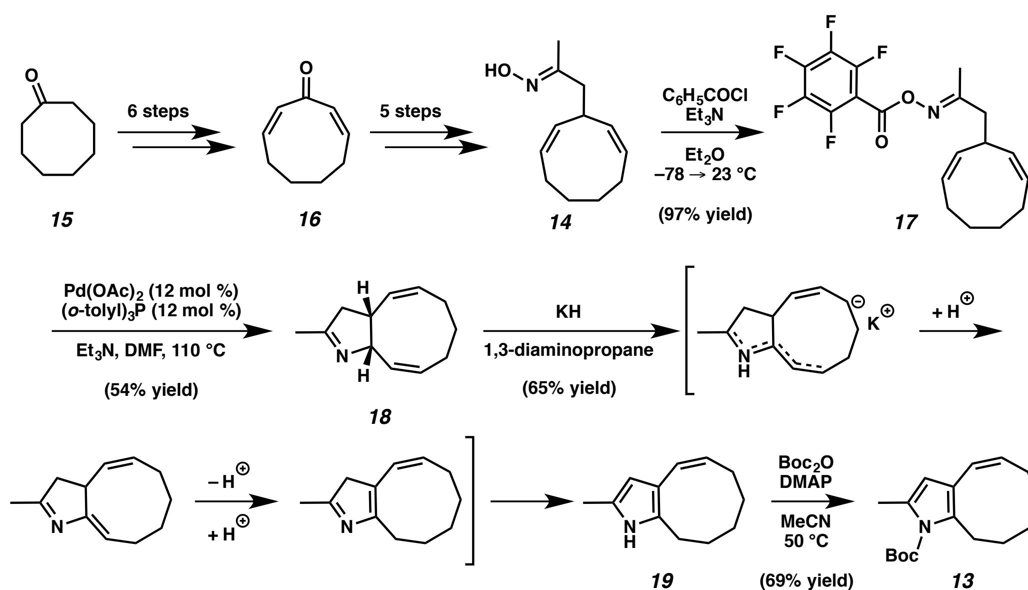
Produced by various strains of the *Serratia* and *Streptomyces* bacteria,[29] the prodigiosin alkaloids have attracted great interest due to their potential as immunosuppressive agents for organ transplants[30] and as promising anticancer agents.[31] Aiming to settle a decade-long structural disagreement among isolation chemists[32] while illuminating the biological profile of a less abundant member of the natural product family, Fürstner and co-workers embarked on a total synthesis of butylcycloheptylprodigiosin (**10**).[33] They envisioned accessing the natural product through cross-coupling of triflate **11**, which would also serve as a scaffold from which to generate prodigiosin analogs through treatment with various cross-coupling partners (Scheme 1.1) Key intermediate **11**, in turn, could be prepared from aldehyde **12**, which would require an intricate sequence of transformations for assembly due to challenges associated with the inherent strain of the *ortho*-pyrrolophane core. Noting the thermodynamic and kinetic disfavorability of nine-membered rings,[34] Fürstner and co-workers opted for a strategy that assembled the carbocycle as soon as possible. As such, they planned to access aldehyde **12** via oxidation of bicycle **13**, which could be prepared through an "aza-Heck" cyclization of oxime **14** similar to that pinoeered by Narasaka and co-workers.[35]

*Scheme 1.1 Fürstner's retrosynthetic analysis of butylcycloheptylprodigiosin (**10**)*



In the forward direction, Fürstner and co-workers converted cyclooctanone (**15**) to (*Z*,*Z*)-cyclononadienone (**16**) over six steps and next accessed oxime **14** through a five-step sequence (Scheme 1.2). Treatment of **14** with pentafluorobenzoyl chloride afforded **17**, the substrate for the key Narasaka–Heck cyclization. Gratifyingly, cyclization occurred smoothly and was viable on multigram scale. Surprisingly, however, pyrrole formation was not observed, with bicyclic imine **18** arising as the major product instead. To induce aromatization, Fürstner and co-workers adopted a thermodynamic deprotonation/reprotonation procedure mediated by potassium hydride. The resulting labile pyrrole (**19**) was immediately *N*-protected, forming bicyclic intermediate **13**.

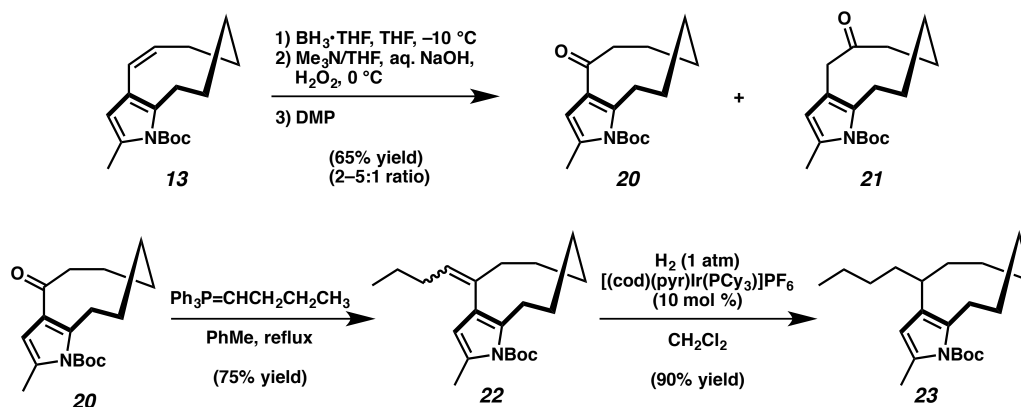
*Scheme 1.2 Preparation of bicyclic intermediate **13***



With bicyclic pyrrole **13** in hand, Fürstner and co-workers proceeded to install the butyl side chain onto the carbocyclic framework by way of alkene oxidation followed by Wittig olefination and hydrogenation. Unfortunately, attempts to oxidize the olefin in the nine-membered ring by means of Wacker oxidation,[36] rhodium-catalyzed hydroboration, or oxymercuration proved unsuccessful. Finally, stoichiometric hydroboration using BH_3•THF followed by stepwise oxidation with H_2O_2 and subsequent Dess–Martin oxidation[37] enabled access to ketone **20** along with undesired isomer **21** (Scheme 1.3). Separation of the two isomers by flash chromatography enabled **20** to serve as the platform for the endgame strategy. Interestingly, Wittig olefination of ketone **20** was only possible in refluxing toluene, which was attributed to steric shielding of the carbonyl moiety. The resulting mixture of *E* and *Z* geometric isomers of alkene **22** was then

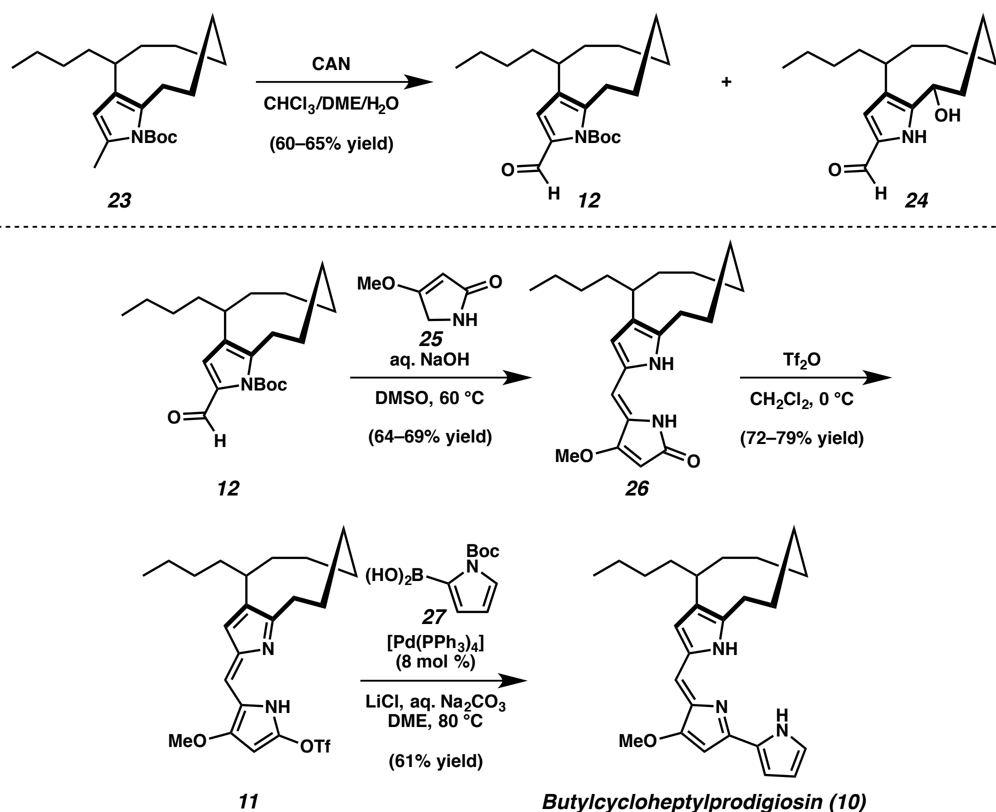
treated with Crabtree's catalyst[38] under hydrogen atmosphere, effecting regioselective hydrogenation to furnish compound **23** in excellent yield.

Scheme 1.3 Introduction of the n-butyl substituent into the carbocyclic framework



Completion of the total synthesis of **10** was achieved in four steps from pyrrole **23**. While initial efforts to oxidize **23** using standard conditions with cerium ammonium nitrate (CAN) proved unsuccessful, careful optimization revealed that use of dimethoxyethane (DME) as the reaction solvent was critical. Under these conditions, oxidation occurred smoothly, furnishing desired aldehyde **12** in good yield along with over-oxidation product **24**, which was readily removed through flash chromatography (Scheme 1.4). Base-promoted aldol condensation of **12** and commercially available lactam **25** with concomittant removal of the Boc protecting group afforded compound **26**, and subsequent treatment with triflic anhydride induced π-system reorganization to supply vinyl triflate **11**. The final Suzuki coupling was carried out using boronic acid **27**, catalytic [$Pd(PPh_3)_4$], and superstoichiometric LiCl under previously optimized conditions,[39] delivering the prodigiosin **10** in 23 steps overall from cyclooctanone.

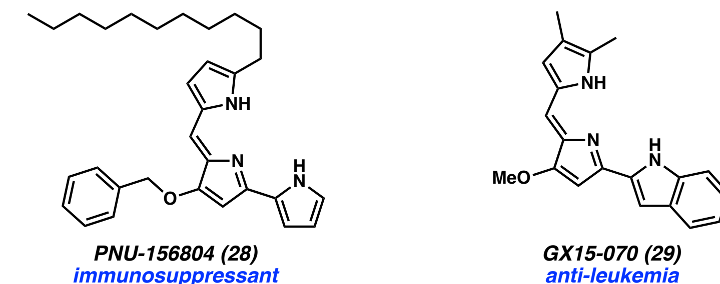
*Scheme 1.4 Completion of the total synthesis of butylcycloheptylprodigiosin (**10**)*



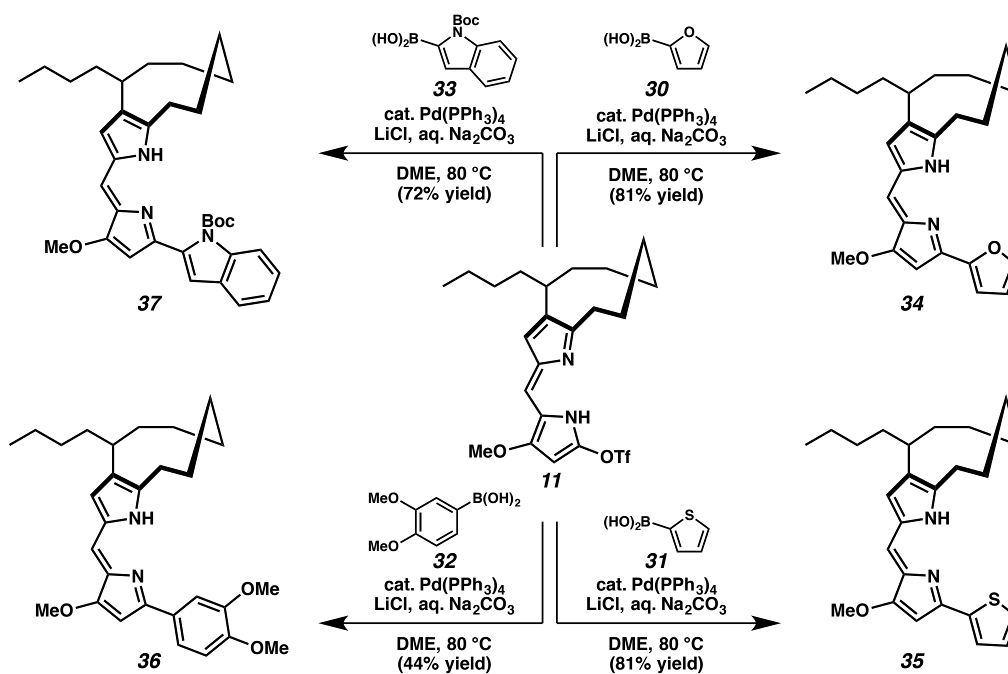
Having accomplished the total synthesis of **10** and reaffirmed the structure proposed by the original isolation chemists, Fürstner and co-workers turned their attention to the diversification of late-stage intermediate **11**. Given the therapeutic properties of simplified prodigiosin analogs PNU-156804 (**28**), which was shown by in vivo studies to act as an immunosuppressant,[30] and GX15-070 (**29**), which was recently advanced into phase I/II clinical trials for treatment of refractory chronic lymphoid leukemia (Figure 1.3),[40] Fürstner and co-workers surmised that variation of the final cross-coupling partner with **11** could generate a variety of biologically active prodigiosin analogs. To this end, triflate **11** was treated with boronic acid derivatives **30–33** under the same cross-coupling

conditions employed in the synthesis of **10**, generating analogs **34–37** in good to excellent yields (Scheme 1.5).

Figure 1.3 Simplified prodigiosin analogs exhibiting therapeutic properties



*Scheme 1.5 Diversification of intermediate scaffold **11***



While the natural prodigiosins display nuclease-like activity, inducing oxidative DNA cleavage,[29] incubation of the non-natural prodigiosin analogs **34–37** with purified double-stranded plasmid DNA of the bacteriophage *Φ*X174 in the presence of $Cu(OAc)_2$ resulted

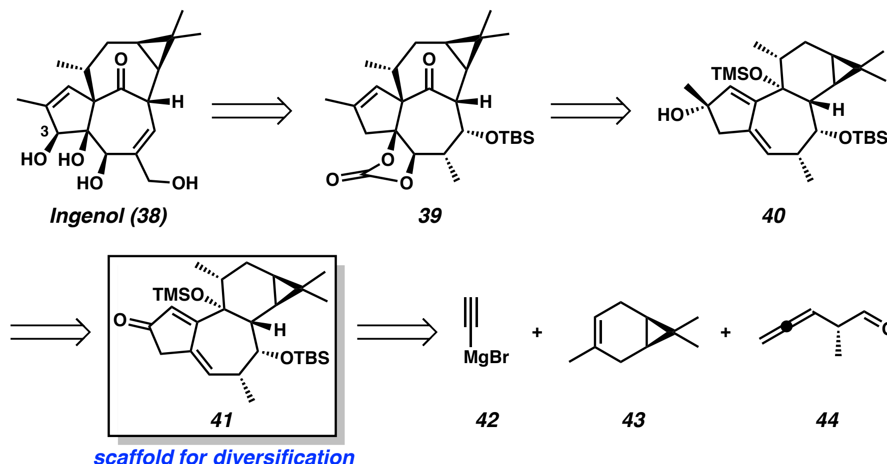
in a distinct lack of nuclease ability in any of the synthetic analogs, as indicated by agarose gel electrophoresis.[41] Notably, under the same conditions, prodigiosin **10** effected single-strand DNA cleavage, in accordance with previous studies. Based on these observations, Fürstner and co-workers concluded that the terminal pyrrole present in the natural prodigiosins is critical to the biological potency of the compounds, as formal replacement with other electron-rich arenes resulted in loss of nuclease activity despite the similarity in overall electronic distribution within the heterocyclic perimeter.

1.3.1.2 BARAN'S INGENOL SYNTHESIS

Polyoxygenated terpenoid natural products are potent biological agents in a variety of therapeutical areas, including oncology, immunology, and infectious diseases.[42] Due to the challenges associated with obtaining these compounds from their natural sources,[43] many synthetic chemists have targeted these important molecules in total synthesis research programs.[44] In 2013, Baran and co-workers completed the total synthesis of ingenol (**38**),[45] a plant-derived diterpenoid featuring a unique [4.4.1]bicycloundecane core.[46] Encouraged by the anticancer and anti-HIV activities displayed by ingenol esters,[47] the Baran group entered into a collaborative effort with LEO Pharma, the producer of the pharmaceutical known as Picato (ingenol metabutate), an FDA-approved treatment for actinic keratosis, a pre-cancerous skin affliction.[48] Under the auspices of this industrial–academic collaboration, the Baran group designed a synthetic route to ingenol (**38**) with two explicit goals: 1) brevity for the sake of commercial viability and 2) amenability to the production of analogs.

Taking cues from biosynthesis[49] and past synthetic studies,[50] Baran's retrosynthetic analysis of **38** accesses the natural product through allylic oxidation and deprotection of carbonate **39**, which would be assembled via stereoselective dihydroxylation and vinylogous pinacol rearrangement of **40**. Tetracycle **40** could be prepared through Grignard addition to **41**, the complex intermediate which would later serve as a scaffold for diversification studies. This core structure could be constructed readily from ethynyl magnesium bromide (**42**), commodity chemical (+)-3-carene (**43**), and aldehyde **44** (Scheme 1.6).

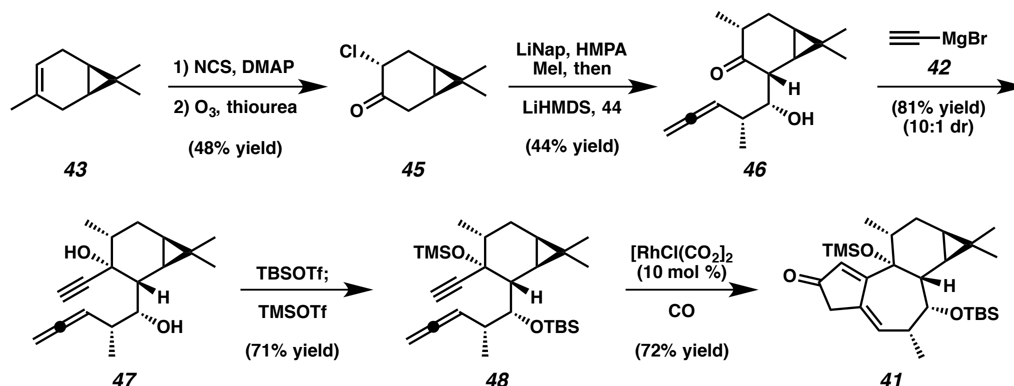
*Scheme 1.6 Baran's retrosynthetic analysis of ingenol (**38**)*



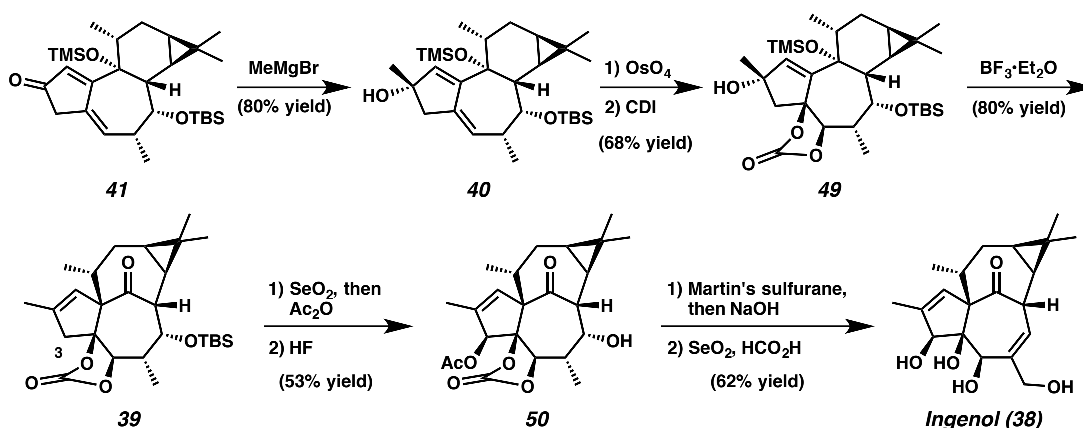
The forward synthesis began with chlorination and ozonolysis of **43** to generate ketone **45**, followed by tandem methylation and aldol reaction with aldehyde **44** to access allene compound **46** (Scheme 1.7). Addition of ethynlmagnesium bromide (**42**) furnished diol **47**, which was treated sequentially with TBS triflate and TMS triflate to incur stepwise protection of the two hydroxyls, thereby suppressing undesired reactivity in the

subsequent transformation. Subjection of bis-protected diol **48** to conditions for allenic Pauson–Khand cyclization[51] resulted in formation of the key tetracyclic intermediate, **41**.

Scheme 1.7 Assembly of core scaffold ***41***



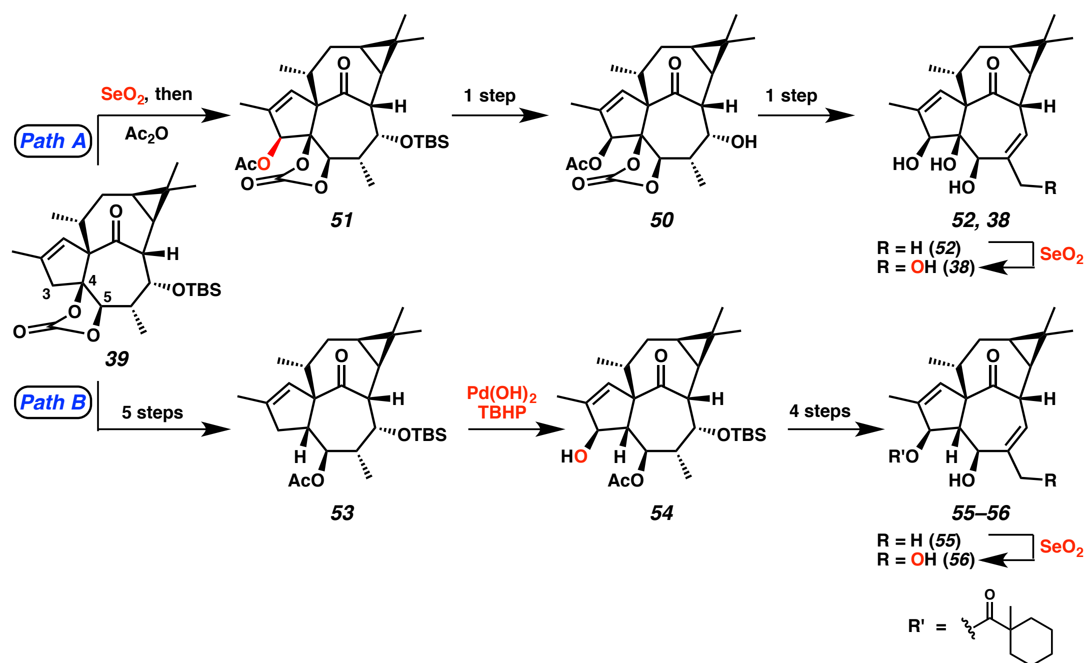
*Scheme 1.8 Completion of the total synthesis of ingenol (****38****)*



Key intermediate **41** was advanced to carbonate **49** by methylation to produce alcohol **40**, followed by osmium-mediated hydroxylation and protection using *N,N*-carbonyldiimidazole (CDI) (Scheme 1.8). After numerous efforts to induce the key vinylogous pinacol rearrangement of **49**, Baran and co-workers found that treatment with BF_3•Et_2O at low temperature effected the desired transformation, assembling the

rearranged core structure **39** in high yield. Subsequent allylic oxidation by SeO_2 and acylation followed by alcohol deprotection delivered acetate **50**. Completion of the total synthesis was achieved through concomittant global deprotection and alcohol elimination using Martin's sulfurane, followed by allylic oxidation using Shibuya's conditions to avoid overoxidation.[52] Overall, Baran and co-workers accomplished the total synthesis of ingenol (**38**) in 14 steps and 1.2% overall yield from **43**.

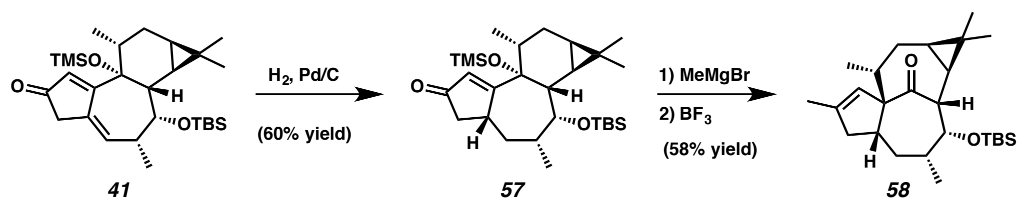
*Scheme 1.9 Oxidative diversification of scaffold **39** (four steps from core scaffold **41**)*



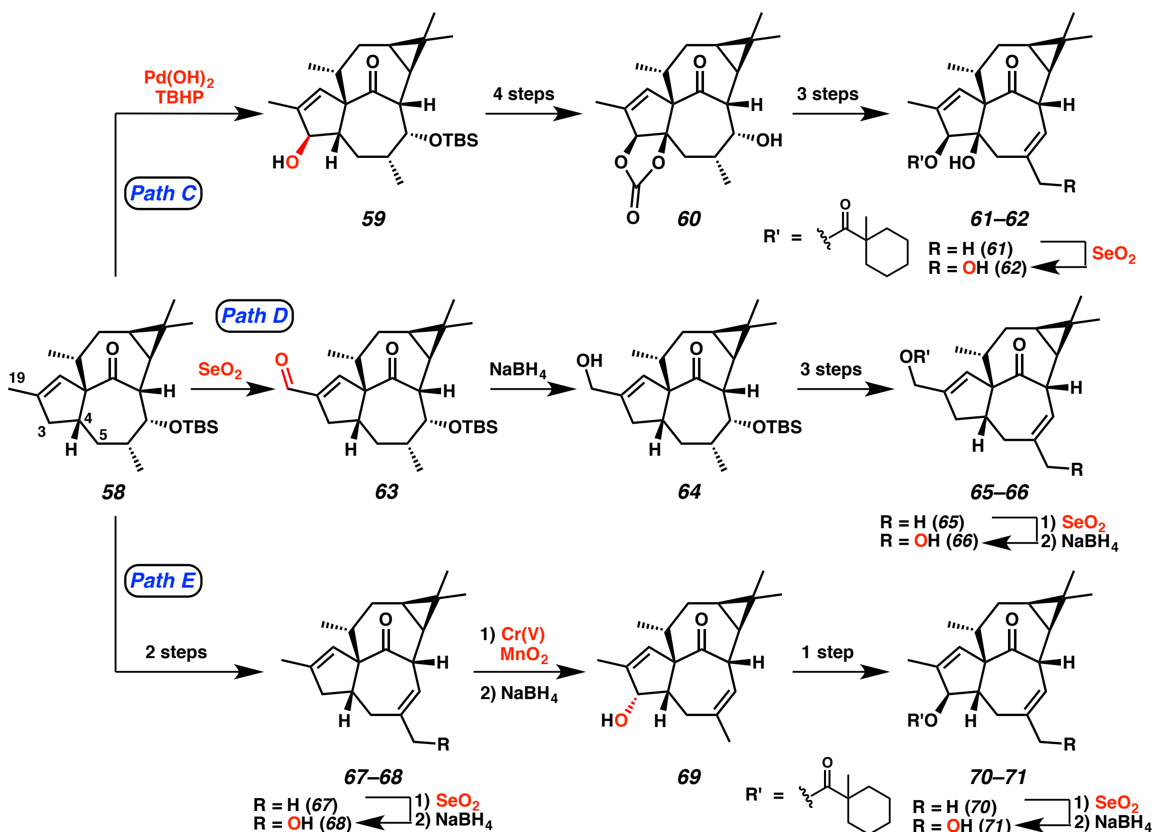
Having met their first goal of crafting a concise synthesis of **38**, Baran and co-workers set their sights on the second goal of preparing ingenane analogs for biological evaluation.[53] Specifically, they aimed to systematically assess the role of the four hydroxyl groups in the biological profile of **38**. To this end, they carried out a series of transformations on carbonate **39**, the preparation of which was greatly facilitated by the

development of a catalytic protocol for the previously stoichiometric osmium dihydroxylation of **40**. Two main pathways for diversification were pursued: Path A involved elaboration to 20-deoxyingenol (**52**) and ingenol (**38**) while Path B entailed the preparation of 4-deoxyingenanes **55** and **56** (Scheme 1.9).

*Scheme 1.10 Elaboration of core scaffold **41** into scaffold **58***



*Scheme 1.11 Oxidative diversification of scaffold **58** (three steps from core scaffold **41**)*



To access less-oxidized analogs, Baran and co-workers elaborated scaffold **41** into **58** by way of regioselective hydrogenation followed by Grignard addition and vinylogous pinacol rearrangement (Scheme 1.10). Interestingly, treatment of **58** with $Pd(OH)_2$ and *tert*-butyl hydroperoxide (TBHP)[54] resulted in oxidation at the C3 position to form allylic alcohol **59**, whereas treatment with SeO_2 effected oxidation at the C19 position, generating aldehyde **63** (Scheme 1.11). This divergency in reactivity formed the basis of Paths C and D, which led to the production of 5-deoxyingenanes **61–62** and **65–66**, respectively. A third pathway, Path E, was accessible through alcohol deprotection and subsequent dehydration to form diene **67**. Curiously, **67** proved unreactive under the $Pd(OH)_2$/TBHP conditions used for C3 oxidation in Path C but underwent C3 oxidation with the opposite facial selectivity when subjected to the Baran group's recently developed Cr(V)-based conditions,[55] generating allylic alcohol **69**. Stereochemical inversion at C3 was accomplished using a Mitsunobu reaction, enabling access to analogs **70** and **71**.

With the ingenol analogs in hand, Baran and co-workers investigated the ability of these compounds to activate human recombinant protein kinase C (PKCδ), stimulate IL-8 release in primary epidermal keratinocytes, and induce oxidative burst in polymorphonuclear leukocytes (neutrophils) based on a previously developed screening cascade. The PKC enzymes play an essential role in mediating cell metabolism, growth, and apoptosis. The PKCδ isoform has been indicated as a tumor suppressant in keratinocytes[56] and is necessary for the attraction of neutrophils, immune cells essential to the antitumor mechanism of Picato.[57] Collaborative studies with scientists at LEO Pharma revealed that the C4 and C5 hydroxyl moieties are critical to the ability of the

ingenol-based compounds to activate PKCδ and stimulate IL-8 release.[53] While the absence of oxygenation at only one of the two positions resulted in only moderate reduction of potency, deoxygenation at both C4 and C5 resulted in a significant loss in activity, with analogs **66** and **68** exhibiting low or nonexistent activity.

Interestingly, however, the ability to induce neutrophil oxidative burst was not influenced by the oxidation patterns at C4 and C5. Despite its inactivity in the PKCδ and IL-8 assays, analog **66** exhibited high potency in the oxidative burst studies, an unexpected observation due to previously established correlations between PKCδ activation and oxidative burst induction.[58] Through these findings, the authors surmised that a PKC isoform other than PKCδ is operative in oxidative burst induction in neutrophils, a hypothesis that was tested by examining the ability of the ingenol analogs to activate PKCβII. As predicted, analogs **66** and **71** were active in nanomolar concentrations. Together, these studies showcase the potential that natural product-based diversification programs have to offer in guiding total synthesis projects and exploring the structure-activity relationships of these non-naturally occurring molecules.

1.3.2 INDEPENDENTLY DESIGNED NATURAL PRODUCT SCAFFOLD

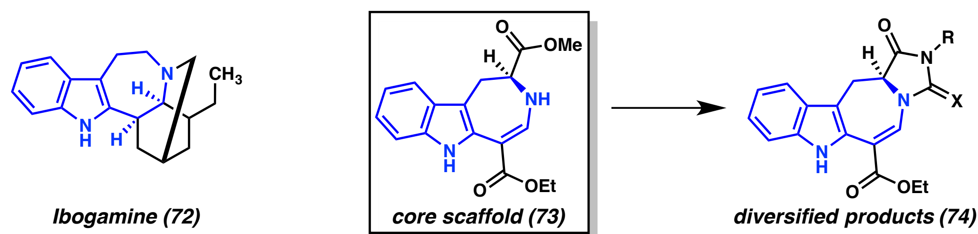
Rather than arising as an intermediate in a total synthesis, an independently designed natural product scaffold is created with the specific intention of use as a starting point for diversification studies. This nuance in research plan may result in subtle discrepancies in functional handles present in an independently designed scaffold as compared to a total synthesis intermediate scaffold. For instance, an independently designed scaffold may strategically include an olefin or carbonyl for use as a versatile diversification handle

when these functionalities might be unnecessary or even detrimental in a total synthesis route and therefore excluded in a late-stage intermediate.

1.3.2.1 SUN'S IBOGAMINE-INSPIRED TETRAHYDROAZEPINO INDOLES

The iboga alkaloid natural products display important biological activities including *N*-methyl-D-aspartate (NMDA) receptor antagonism and opioid (k) receptor agonism.[59] Structurally, the iboga alkaloids feature seven-membered azepino[4,5-*b*]indole ring systems present in various other biologically active natural products.[60] Noting the correlation between the azepino indole framework and biological potency, Sun and co-workers sought to prepare the iboga alkaloid core (**73**) and append a substituted hydantoin motif to access a set of diversified compounds (**74**) (Figure 1.4).[61] Given the biologically privileged nature of hydantoin[62] and the biological activity of the iboga alkaloids, the combination of the two motifs was hypothesized to result in access to therapeutically interesting iboga analogs.

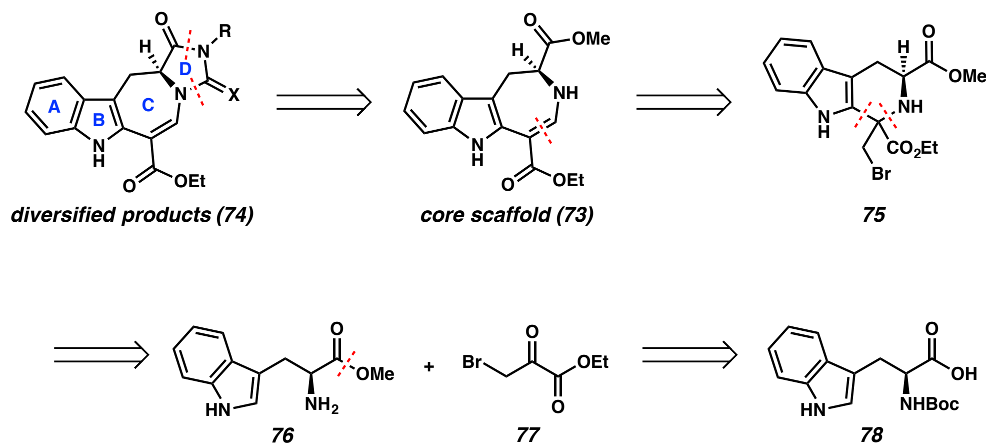
*Figure 1.4 Ibogamine-inspired core scaffold **73** and targeted diversified products **74***



Building on previously reported efforts toward azepino indole scaffolds, Sun and co-workers developed a concise synthetic route toward **73** that avoids several drawbacks of

previously established strategies,[55b,63] including prolonged reaction times, use of toxic reagents, and poor yields. Their retrosynthetic plan involved accessing the diversified hydantoin-fused tetrahydroazepino compounds (**74**) through urea formation from tricycle **73** and subsequent intramolecular cyclization to form the D ring (hydantoin moiety). Core scaffold **73** would be obtained through ring expansion of tricycle **75** (via intramolecular *N*-alkylation and aziridine ring-opening), which could be assembled by Pictet–Spengler condensation of L-tryptophan methyl ester **76** and bromopyruvate **77** (Scheme 1.12).

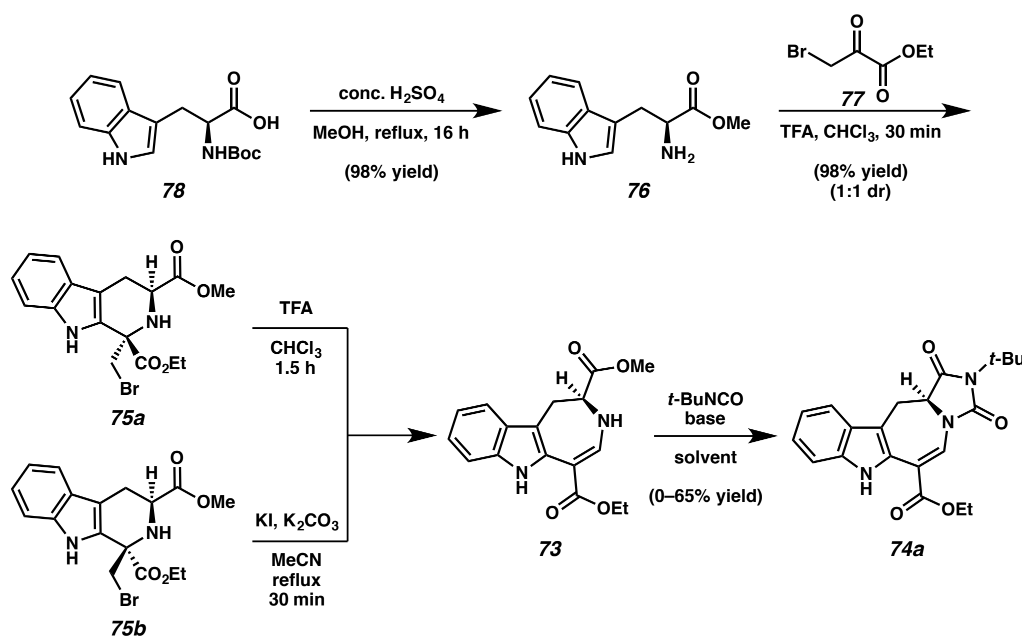
*Scheme 1.12 Sun's retrosynthetic analysis of hydantoin-fused tetrahydroazepino compounds **74***



Preparation of core scaffold **73** was achieved rapidly, beginning with esterification of L-tryptophan (**78**) followed by Pictet–Spengler condensation with bromopyruvate **77**, with both transformations proceeding in excellent yield. Although the tricyclic product of the Pictet–Spengler reaction was formed as a 1:1 mixture of diastereomers, both isomers were efficiently converted into scaffold **73**, albeit under drastically different conditions. Interestingly, the (*1S,3S*) diastereomer **75a** rearranged readily to **73** under

acidic conditions at ambient temperature, whereas the (*1R,3S*) diastereomer **75b** required refluxing basic media to undergo the desired transformation. With key scaffold **73** in hand, Sun and co-workers proceeded to examine the final cyclization event. Disppointingly, initial efforts to effect hydantoin formation using *tert*-butyl isocyanate and various bases in a variety of solvents either proved unsuccessful or resulting in only low yields of desired tetracycle **74a** (Scheme 1.13).

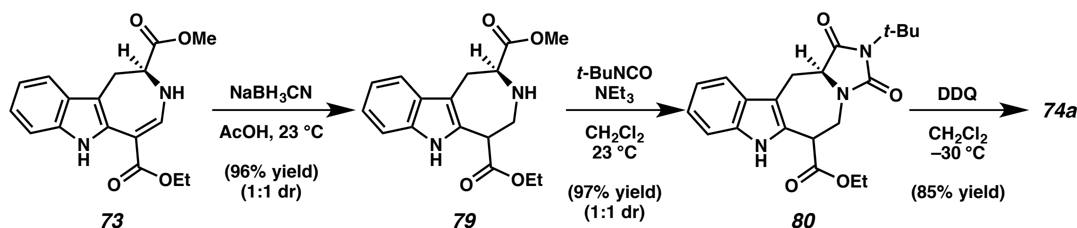
*Scheme 1.13 Preparation of scaffold **79** and initial efforts at product (**74a**) formation*



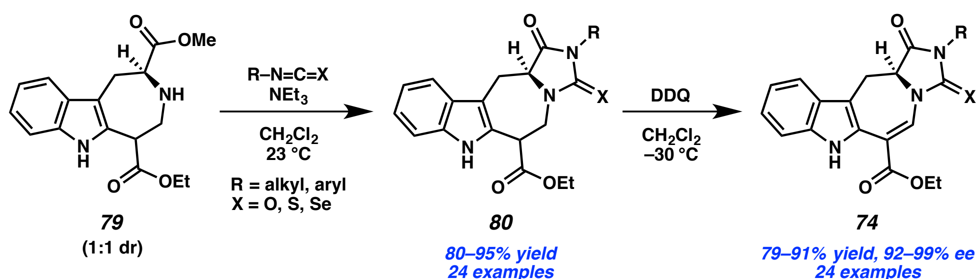
Hypothesizing that delocalization of the nitrogen lone pair through the α,β-unsaturated ethyl ester in **73** was causing low nitrogen nucleophilicity (and therefore low yields of **74**), Sun and co-workers sought to remove the olefinic moiety to facilitate cyclization. Accordingly, treatment of **73** with $NaBH_3CN$ afforded **79** as a mixture of diastereomers which reacted smoothly with *tert*-butyl isocyanate at ambient temperature,

delivering tetracycle **80** as a diastereomeric mixture. After exploration of a number of oxidants, reinstation of the olefin was achieved using DDQ (Scheme 1.14).

*Scheme 1.14 Strategy for accessing tetracyclic product **74a** in higher yield*



*Scheme 1.15 Diversification of scaffold **79** and oxidation to generate varied tetracyclic products **74***



Having elucidated the optimal conditions for this critical sequence of transformations, Sun and co-workers were able to access 24 different tetracyclic compounds (**80**) by using variously substituted isocyanates in the cyclization reaction. Further oxidation of these compounds using DDQ afforded the desired analogs **74** in good to excellent yields (Scheme 1.15). Although the authors do not comment on the biological activities of the compounds generated from these investigations, this contribution provides a good example of strategy for diversifying a natural product-inspired scaffold prepared explicitly for the purposes of creating a library of complex molecules, rather than en route to a total synthesis.

1.3.3 DIVERSIFICATION TO PRODUCE NATURAL PRODUCT HYBRIDS

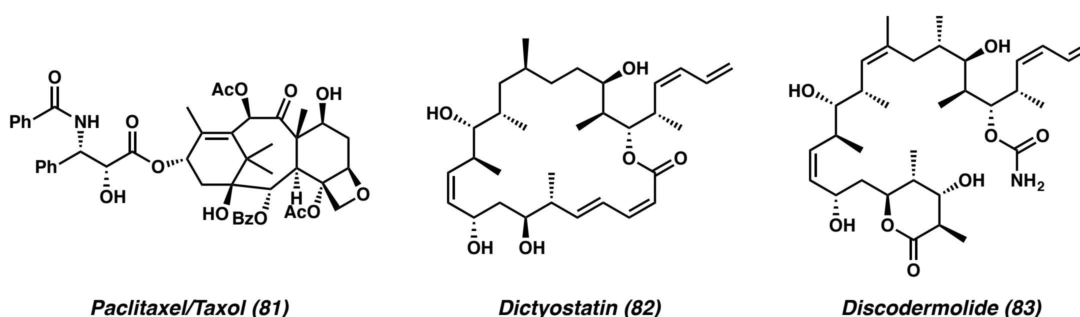
Unique from the previously discussed guiding principles for diversification programs is the strategy of producing hybrid molecules that contain structural features from two or more different families of natural products. With more than one natural product class to inspire diverse structural design, the possibilities are vast for generating derivatives with a wide range of biological activities.

1.3.3.1 PATERSON'S DICTYOSTATIN/DISCODERMOLIDE HYBRIDS

Cancers are among the foremost causes of death in the developed world, and as such, a great deal of effort has been invested in developing treatments for these devastating afflictions. Decades of research have shown that the study of natural products effective in attenuating cell growth through cellular microtubule inhibition is a viable approach toward diminishing the effects of cancer.[64] Indeed, after its discovery in 1962, the diterpenoid natural product paclitaxel (**81**, Figure 1.5)[65] proved to be a competent chemotherapeutic, gaining FDA approval as the pharmaceutical known as Taxol in 1992 and enjoying widespread clinical use.[66] Unfortunately, the taxane class of cytotoxic drugs tend to suffer from low solubility in aqueous media and the rise of drug resistance in patients, ultimately impeding their efficacy as cancer treatments.[67] Given promising leads in the study of cellular microtubule inhibition, there has been a surge of interest among the chemical community in identifying new microtubule-stabilizing agents (MSA) with mechanisms of activity similar to that of Taxol.

While some research groups adopted the strategy of creating direct analogs of Taxol by modifying substituents around the taxane core,[68] Paterson and co-workers took a different approach. Noting that the marine sponge-derived polyketides dictyostatin (**82**)[69] and discodermolide (**83**)[70] share the same microtubule-stabilizing mechanism as Taxol while maintaining efficacy against Taxol-resistant cancer cell lines, Paterson recognized that the two natural products could serve as parent compounds for the design of dictyostatin/discodermolide hyrid molecules.[71] While discodermolide had been synthesized[72] and deemed unfit for clinical use due to pulmonary toxicity revealed in a Phase I clinical trial by Novartis,[73] Paterson envisioned that blending structural features of discodermolide with those of dictyostatin could result in the production of uniquely active therapeutics.[74]

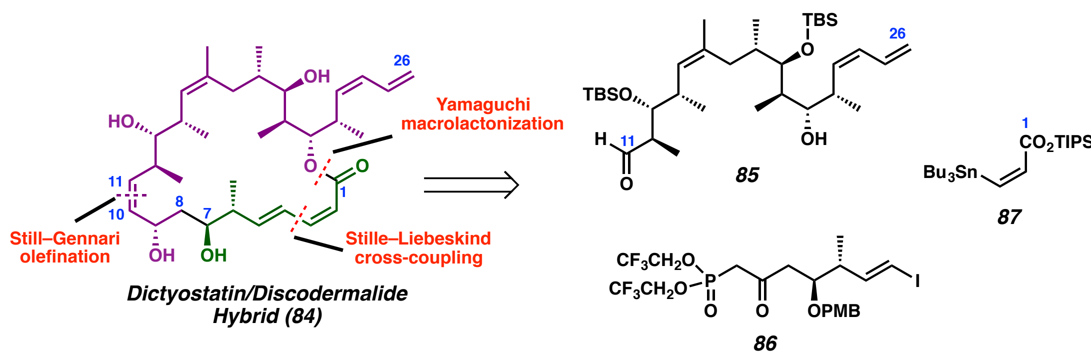
Figure 1.5 Natural products exhibiting microtubule-stabilizing activity



Based on previous investigations by the Canales group into the conformations of paclitaxel, discodermolide, and dictyostatin at the taxoid binding site,[75] Paterson designed dictyostatin/discodermolide hybrid **84**. Canales's studies indicated that structural similarities between discodermolide and dictyostatin corresponded with the three-dimensional regions of greatest overlap in the taxoid binding site. Furthermore, the most

significant spatial discrepancies arose from the δ-lactone and dienoate moieties in discodermolide and dictyostatin, respectively. Because dictyostatin exhibited superior biological activity,[76] Paterson opted to furnish the regions of greatest difference (C1 to C7) with structural features from dictyostatin (highlighted in green) while modeling the regions of closest overlap (C8 to C26) after discodermolide (highlighted in purple) (Scheme 1.16). In doing so, Paterson sought to capture the bioactive potency of dictyostatin while retaining the advantageous binding properties shared by both natural products. Retrosynthetically, Paterson envisioned assembling the macrocyclic core of hybrid **84** using Still–Gennari olefination of known compounds **85** and **86** to form the C10–C11 alkene followed by a cross-coupling/macrolactonization event.

*Scheme 1.16 Paterson's retrosynthetic strategy for dictyostatin/discodermolide hybrid **84***

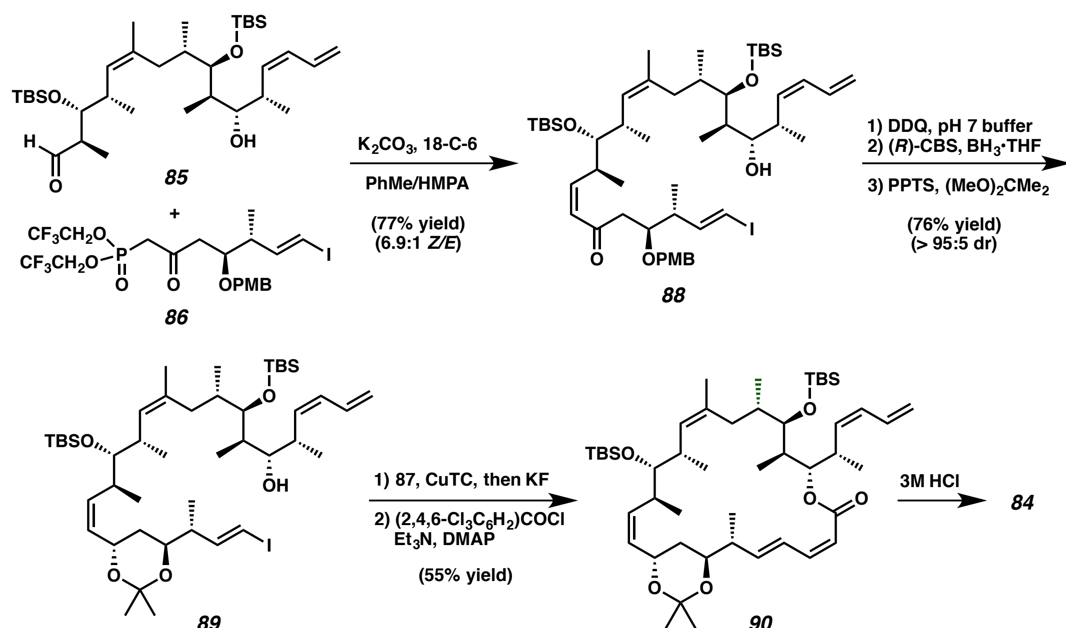


Preparation of hybrid **84** began with previously optimized Still–Gennari olefination of **85** and **86**, which proceeded in good yield and selectivity. Cleavage of the PMB ether using DDQ followed by stereoselective CBS reduction of the enone and acetonide protection afforded vinyl iodide **89** in good yield. Copper-mediated Stille–Liebeskind cross-coupling[77] between **89** and stannane **87** followed by macrolactonization under

modified Yamaguchi conditions[78] delivered the fully protected macrocycle (**90**). Global deprotection in acidic media supplied desired dictyostatin/discodermolide hybrid **84**.

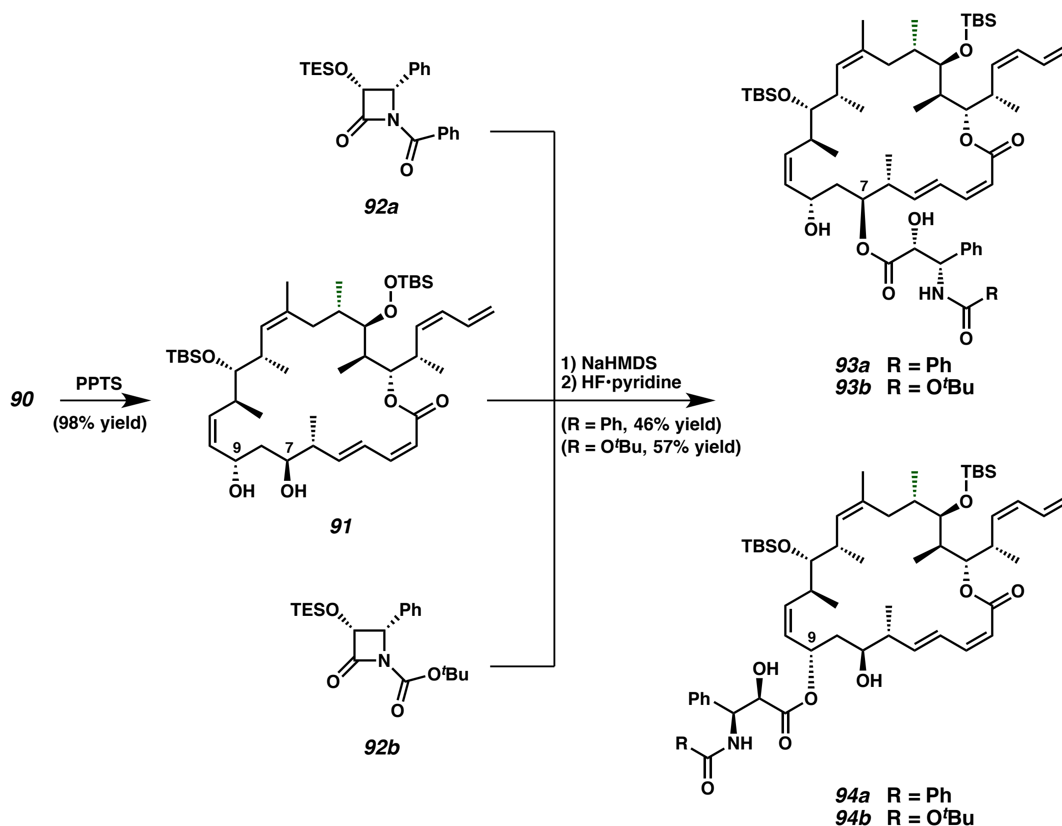
Having prepared hybrid **84** along with several other analogs not highlighted in this discussion, Paterson and co-workers turned their attention to the creation of "triple" hybrids, that is, compounds bearing structural features of three different natural products. Taking cues once again from Canales's binding model of the taxanes, Paterson noted that the C13 side chain of paclitaxel occupies a sizeable pocket of the binding site that remains empty in the discodermolide and dictyostatin binding models. Recognizing that the C7 and C9 hydroxyls of hybrid **84** point in the direction of this pocket, Paterson hypothesized that appendage of the paclitaxel C13 side chains onto the discodermolide/dictyostatin hybrid (**84**) would generate novel triple hybrids that would provide further insights into the binding interactions of the taxanes.

*Scheme 1.17 Synthesis of dictyostatin/discodermolide hybrid **84***



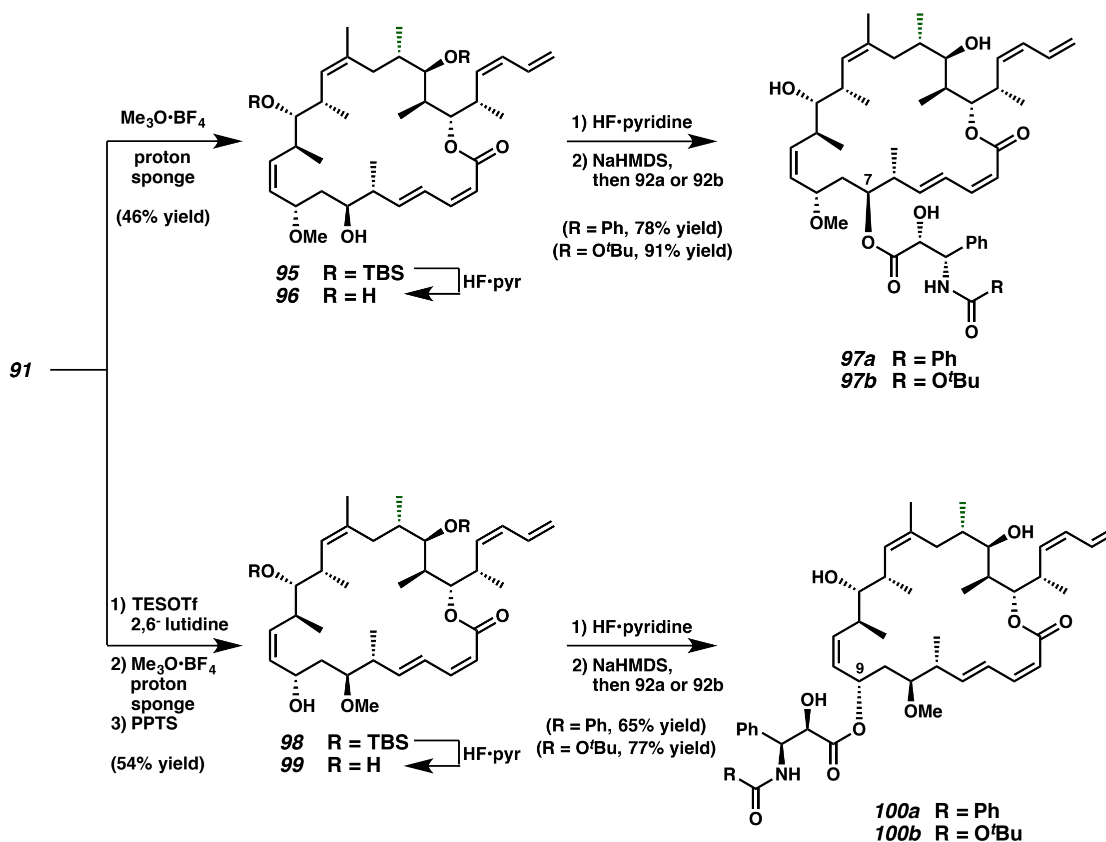
Treatment of fully protected macrocycle **90** with pyridinium *p*-toluenesulfonate (PPTS) effected removal of the acetonide protecting group to furnish 1,3-diol **91**, the scaffold from which an array of triple hybrids would be constructed. In accordance with a previously developed protocol for side-chain introduction,[79] diol **91** was treated with NaHMDS followed by either lactam **92a** or **92b**. The resulting mixture of inseparable C7-esterified and C9-esterified isomers was subjected to TBS deprotection conditions (HF•pyr, pyridine), affording triple hybrids **93** and **94**, which were separated by careful HPLC purification.

*Scheme 1.18 Diversification of scaffold **91** to access "triple" hybrids including Taxol features*



Unfortunately, attempts to characterize these compounds by NMR spectroscopy were hampered by the apparent lability of the newly installed side chains. Paterson and co-workers found that triple hybrids **93** and **94** underwent transesterification in DMSO, producing a mixture of C9 and C7 esters in an approximately 2:1 ratio. Since DMSO is a common solvent for biological assays, these observations invalidated any future biological studies on these hybrids, as any sample would likely contain an isomeric mixture of compounds. The lability of the ester side chains was further highlighted by the regeneration of the original double hybrid **84** when triple hybrids **93** and **94** were allowed to stand as solutions in methanol over 72 hours.

*Scheme 1.19 Preparation of methyl-capped triple hybrids **97** and **100***



To prevent this undesired reactivity without substantially altering the biological profile of the triple hybrids, Paterson and co-workers sought to cap the C9 or C7 hydroxyl as a methyl ether. Selective methylation of the more nucleophilic C9 hydroxyl of **91** using Meerwein's salt and proton sponge enabled access to methyl ether **95**, and subsequent TBS deprotection and esterification with either lactam **92a** or **92b** afforded the C7-esterified triple hybrid **97a** or **97b**, respectively. Access to the C9-esterified triple hybrids **100a** and **100b** was achieved through regioselective C9-silylation, followed by methylation of the C7 hydroxyl and TES deprotection to generate methyl ether **98**. Once again, TBS deprotection and esterification with either lactam **92a** or **92b** delivered triple hybrid **100a** or **100b**, respectively.

With an abundance of taxane derivatives in hand, Paterson and co-workers proceeded to investigate the biological profiles of the hybrid molecules. To this end, they compared the activities of the double and triple hybrid molecules to those of the parent compounds (taxol, discodermolide, and dictyostatin) in assays against human cancer cell lines AsPC-1 (pancreatic), DLD-1 (colon), PANC-1 (pancreatic), and NCI/ADR-Res (taxol-resistant ovarian). These studies revealed double hybrid **84** and its structural derivative, 9-methoxy analog **96**, to be the most potent of all the synthetic compounds, with both exhibiting low nanomolar cytotoxicities in taxol-sensitive and taxol-resistant cell lines. With an IC_{50} value between that of discodermolide and dictyostatin across all cell lines, hybrid **84** was identified as a promising lead compound for further diversification studies. Notably, none of the triple hybrids displayed appreciable cytotoxicity, indicating that the addition of the side chains did not enhance tubulin-binding ability.

1.4 CONCLUSIONS

The diversification of complex scaffolds contributes a wealth of knowledge to the chemical community, as attested to by the surge in enthusiasm for these types of research programs over the last two decades. The contributions of diversification studies to chemical science are twofold. From a synthetic perspective, the preparation of complex scaffolds for diversification often reveals unexpected patterns of reactivity, inspiring methods development and synthetic insight from which future researchers are likely to benefit. Additionally, the observed reactivity of a complex scaffold under established conditions for various transformations contributes valuable information for practitioners of complex molecule synthesis. From a biological perspective, the creation of myriad compounds resembling biologically active complex molecules enables detailed study of structure-activity relationships, systematically increasing the collective understanding of medicinal chemistry and ultimately leading to the next major therapeutic breakthrough. Given these considerable motivating factors, it is likely that diversification projects will soon become mainstays of most synthesis-oriented research programs.

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