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Convergent Synthesis of Anaephene B

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Abstract

Many organisms are capable of producing organic compounds as secondary metabolites; these natural products can be quite pharmaceutically and medicinally relevant.[1] Typically, the more unique an organism, the more unique its secondary metabolites. Cyanobacteria, an abundant microalgal organism, is capable of producing a myriad of novel natural products.[2] The unique photosynthetic and autotrophic properties of cyanobacteria allow for the production of many secondary metabolites, including lipopeptides, amino acids, fatty acids, macrolides, amides, and others.[2,3] *Staphylococcus aureus* is one the most common bacterial infections in the world.[4] *S. aureus* produces a number of metabolites that inhibit host immune responses.[4] As a result, *S. aureus* has become extremely resistant to not only host immune responses, but antibiotics as well.[5] A recent discovery of three alkylphenols coined anaephenes A-C derived from cyanobacterium genus *Hormoscilla* found in Guam has prompted further research into their medicinal relevance.[6] The structure of anaephene B is slightly different from its sister compounds with a higher degree of unsaturation due to a terminal alkyne.[6] Of the three compounds, anaephene B is noted for its distinct antibacterial functioning towards *Staphylococcus aureus*; its synthetic replication and manipulation is the focus of this research.[6]

Introduction

Total Synthesis

Nature is a keen and creative organic chemist; various plants, animals, and microorganisms are responsible for synthesizing a multitude of compounds with abundant pharmaceutical applications. Natural products are compounds derived from living organisms through unique biochemical reactions and pathways.[1] Many of these toxic compounds serve distinct defensive, communicative, and predative functions for microorganisms.[7] Naturally derived compounds have been used for centuries in medicine; records of the use of plants and marine life to treat various illnesses trace back to ancient times.[8] These natural products are extracted through long arduous processes—often yielding only single digit milligram quantities—and tested for advantageous activity through biochemical assays.[9] Naturally derived compounds are often found to exhibit antibacterial, antifungal, and anticancer activity, showing promise for human use.[1] Therefore, once a natural product is identified, the focus shifts to the biochemical characterization and synthetic replication of the molecule. Medicinal activity of natural products is historically tested through a "trial and error" process.[8] This approach, however, causes great difficulty in regards to large-scale reproduction of the compound.

The advancement of pharmaceutics is largely dependent on organic chemistry. The connection between a chemical structure and its biological activity sets the premise for the field of medicinal chemistry.[10] Understanding the specific biomolecular mechanisms of various drugs allows for the development of novel medications.[10] Once a drug is developed, its efficacy and safety is discerned through a series of clinical studies in order to be launched as a legitimate treatment.[10] Many medicinal chemists and drug developers have an advanced

background in synthetic and organic chemistry.[10] Optimization of the organic synthesis of natural products is a major contributor to the field of medicinal chemistry. Total synthesis of complex molecules is a subdiscipline of organic chemistry specifically comprised of naturally derived compounds.[11] The isolation and structural discernment of natural products allows for the synthetic replication of these products.[11] The synthesis of aromatic compounds is notable, as many biologically active derivatives of benzene can be replicated.[11] Modern chemistry techniques allow for the synthesis of very complex molecules through the use of carbon-carbon bond formations and stereospecific reactions.[11] Complex molecular structures often lend to rich biological application; typically, the more unique the molecular structure of a compound, the more unique its source.[12]

Cyanobacteria

Many algal species, specifically cyanobacteria, are recently acknowledged for their production of novel natural products and relevance in medicine. The extraction and synthetic production of natural products is a powerful approach to drug development, specifically antibacterial and antitumor therapies.[1] Production of natural products through synthetic means allows for the understanding, manipulation, optimization and eventual clinical implementation of different drug treatments. While most commonly attributed with toxicity, cyanobacteria and their chemical isolates offer a vast opportunity for pharmaceutical application.

Cyanobacteria are a photosynthetic algal species found in a variety of marine environments across the world.[2] Excessive algal blooms release natural products including cyclic peptides and alkaloids, often toxic to humans and other mammillary species.[9] Natural products produced by these microorganisms were historically considered waste; however,

many of these toxic compounds serve distinct defensive, communicative, and predative functions.[7] The natural products released by cyanobacteria support their resilience and extensive colonization, quite possibly leading to their relevance in medicine.[2] The expansive nature of cyanobacteria's secondary metabolites allow for the production of a wide range of bioactive molecules: lipopeptides, amino acids, fatty acids, macrolides, amides, and others.[3] The uniqueness of the prokaryotic cyanobacteria cells establishes their abundant medicinal qualities, including their photosynthetic, nitrogen fixation, and autotrophic properties.[2] Understanding the principles of why these compounds are produced and how they are used to promote cyanobacteria survival can aid in drug development. A higher percentage of bioactive natural products extracted from cyanobacteria have been identified as novel molecules compared to a relatively high rediscovery rate utilizing traditional natural product sources (**Figure 1**).[12] Thus, cyanobacteria offer an avenue for both complex and simple natural product discovery that may be used in furthering the development of associated medications.

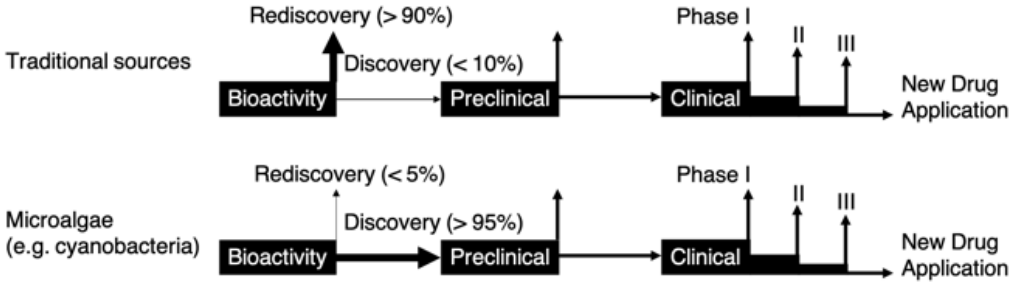


Figure 1. The percent discovery of novel natural products from traditional sources compared to microalgae.[12]

Cryptophycins, a 16 membered family of antimitotic agents, are among the many natural products isolated from cyanobacteria found to exhibit anticancer potential.[13] Isolated from the *Nostic* cyanobacterial species, cryptophycins are a successful cytotoxic treatment for tumor cells resistant to current therapies.[13] Cryptophycin-1 works by inhibiting tubular

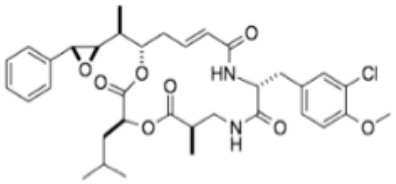


Figure 2. Chemical structure of cryptophycin-1.[14]

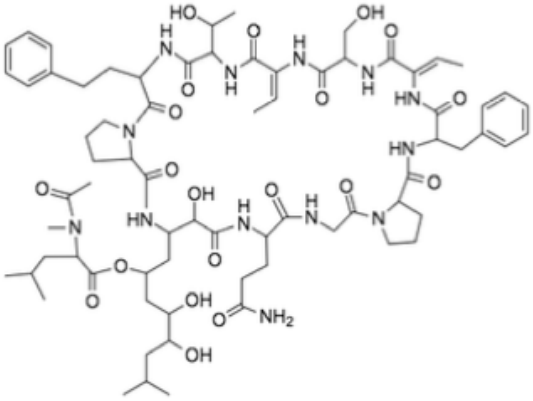


Figure 3. Chemical structure of pahayokolide A.[3]

polymerization, halting the cell cycle at the G2//M phase and triggering apoptosis (**Figure 2**).[14] It was found that as little as six hours after treatment with cryptophycin-1, cancer cells were arrested in the mitotic stage of the cell cycle and programmed for cell death by cysteine proteases.[14] The relative speed and specificity of this natural product propels its clinical use and inspires further research into other cyanobacteria derived compounds.

Additional natural products derived from cyanobacteria have been found to exhibit potent antibacterial activity, including pahayokolide A (**Figure 3**).[3] Pahayokolide A is a secondary metabolite derived from *Lyngbya* in the Florida Everglades.[15] The chemical structure of pahayokolide A was determined using mass spectrometry.[15] Antibacterial activity of pahayokolide A was determined using an agar diffusion culture, showing inhibition of *Bacillus megaterium* and *Bacillus cereus.*[16] Pahayokolide A was also found to inhibit the growth of other cyanobacteria, likely to rid of competition for resources and nutrients.[16] Not only do natural products of cyanobacteria aid in their own survival, they show great promise for use in human medicine.

Slight structural differences of cyanobacteria derived natural products can have a significant impact on their bioactivity. For example, cyanopeptolins are one of the most common peptides produced by cyanobacteria and have been extensively studied for their inhibition of serine proteases.[17] In a previous study, cyanopeptolins extracted from the cyanobacterium *Nostoc edaphicum* in the Baltic Sea were tested for their protease inhibition potential.[17] Cyanopeptolins are composed of a six membered amino acid ring, each containing several different amino acid residues.[17] Through an enzyme assay, tyrosine containing cyanopeptolins were found to selectively inhibit chymotrypsin, while arginine containing cyanopeptolins inhibited trypsin only at low levels (**Figure 4**).[17] Although the majority of cyanopeptolins exhibit inhibitory qualities, the presence of distinct amino acid residues and their associated functional groups is imperative to their biological functioning.[17] The functioning of natural products derived from cyanobacteria is extremely dependent on their specific chemical structure.

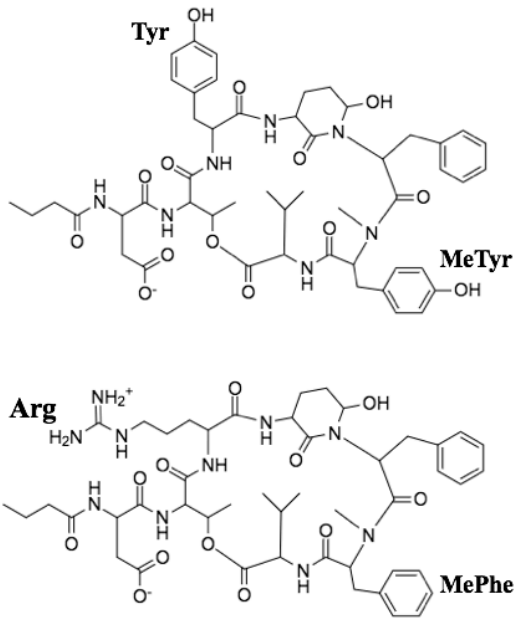


Figure 4. Chemical structures of tyrosine and arginine containing cyanopeptolins.[17]

Staphyloccocus aureus

Staphylococcus aureus, or "staph", is an extremely widespread bacterial infection that affects approximately 50% to 60% of people across the world.[4] Those already infected by *S. aureus* are at increased risk of becoming infected by new strains.[18] From 1998 to 2005, *S. aureus* was the most common bacterial culture in 300 United States clinical laboratories.[4] *S.*

aureus is a gold pigmented, gram-positive bacterial species part of the Micrococcaceae family.[18] Its cell wall consists of 50% peptidoglycan by weight; peptidoglycan is composed of alternating *N*-acetylglusamine and *N*-acetylmuramic acid subunits with 1, 4-β linkages (**Figure 5**).[18] *S. aureus* contains many surface proteins that bind extracellular matrix molecules, including elastin binding and collagen binding proteins, that help the bacteria colonize host tissue (**Figure 5**).[18] *S. aureus* manifests in skin and soft tissue, causing both infections and abscesses.[4] Most infections present as pneumonia or invasive blood stream infections and can be very dangerous.[19] Because of its ability to develop antibacterial and immunological resistance, *S. aureus* is a leading pathogenic cause of morbidity and mortality in humans.[4]

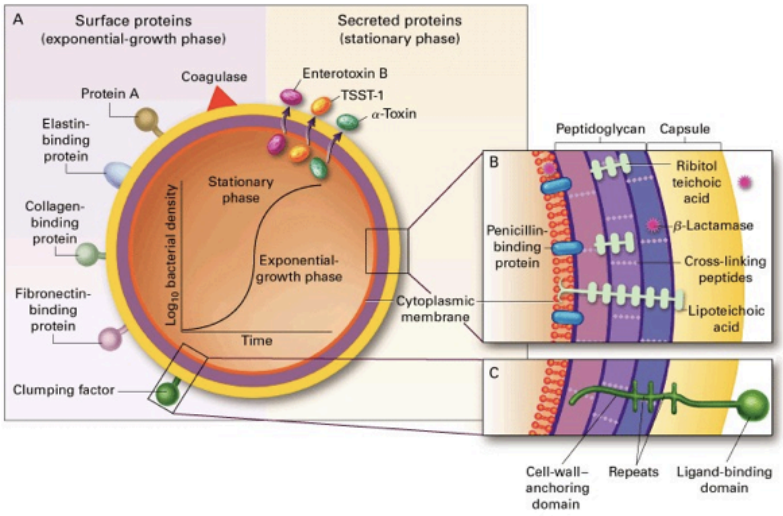


Figure 5. Structure of *S. aureus*.
The spherical shape of *S. aureus* is characteristic of the Micrococcaceae family. Panel A shows the surface proteins of *S. aureus,* including elastin-binding and collagen-binding proteins. Panel B shows the bacterial cell wall, including the peptidoglycan layer.[18]

Pathogenesis & Innate Immune Response

The pathogenesis of *S. aureus* begins with a breach of the skin, allowing for manifestation in soft tissue.[4] It first directly adheres to an endothelial cell and is then ingested by the cell through phagocytosis; proteolytic enzymes are then released to facilitate infection.[4] *S. aureus* is first combatted by the innate immune system, where neutrophils are recruited by cytokines, chemokines, and chemokine receptors.[19] The neutrophils then attack

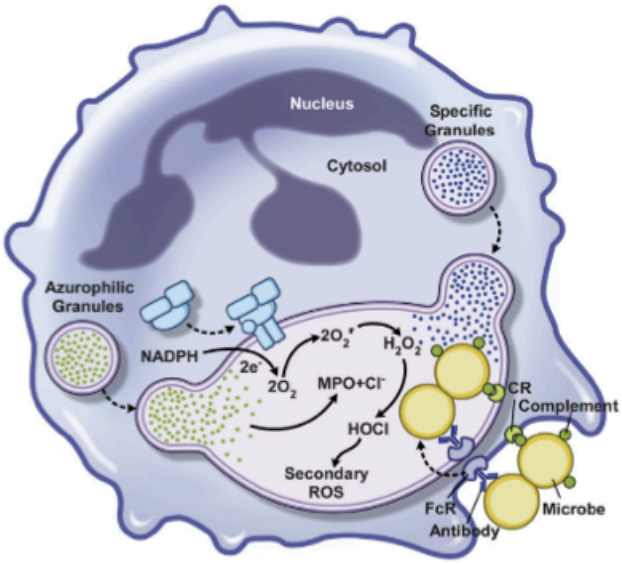


Figure 6. Polymorphonuclear leukocyte phagocytosis and microbicidal processes. Surface receptors for host opsonins, such as complement and anti- body, promote ingestion of S. aureus, which, in turn, activates the microbicidal processes that operate in a bacteria-containing phagosome (the cytoplasmic vacuole containing bacteria). The enzyme complex responsible for generation of superoxide – NADPH oxidase – depicted by the blue cluster of shapes on the phagosome membrane. CR, complement receptor; HOCl, hypochlorous acid; MPO, myeloperoxidase; ROS, reactive oxygen species.[4]

the foreign bacteria through phagocytosis and oxidative killing.[19] Superoxide radical species produced by neutrophils activate NADPH oxidase, which transports electrons into a variety of oxygen metabolites that enhance phagocytotic activity (**Figure 6**).[4] As the infection invades its host cell, keratinocytes detect foreign microbes and signal for an inflammatory response, leading to the formation of an abscess.[4] Abscess formation is governed by polymorphonuclear leukocytes (PMNs).[4] PMNs work by attacking *S. aureus* mechanisms of infection, including its ability to neutralize superoxide radical ions and other radical oxygen species.[4] While adaptive immunological responses have been reported, few suggest an ability to combat future infection.[19] *S. aureus* exhibits a counter attack to the immune system by inducing neutrophil apoptosis, lysing the cell and spreading into surrounding tissue.[19]

Treatment

S. aureus produces a number of metabolites that inhibit host immune response, including N- formylated virulence factors that evade the PMN mechanism.[4] Cytolytic toxins are also secreted by the bacteria at the site of infection that can permeate host membranes and

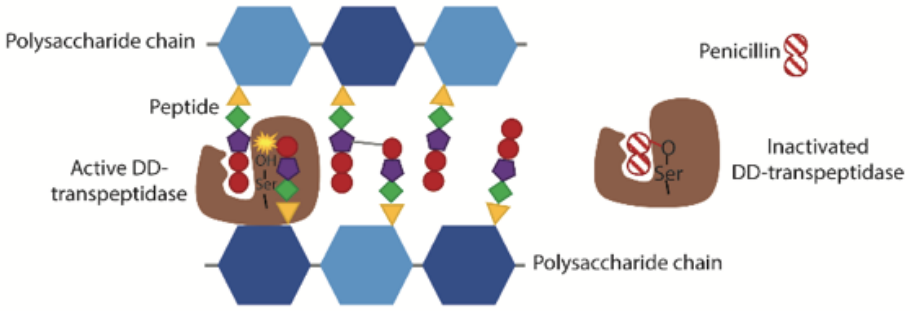


Figure 7. Transpeptidase enzyme (brown) binds two alanine residues (red circles) and catalyzes crosslinking. Penicillin (red stripes) mimics the shape of the alanine residues, binding and inactivating transpeptidase enzyme.[20]

cause cytolysis.[4] As a result, *S. aureus* developed resistance to host immune responses and antibiotics.[5] Penicillin, a commonly prescribed antibiotic, serves as the leading antibiotic used to treat *S. aureus*, among others.[5] Penicillin was discovered in 1928 as an antimicrobial product of the fungal species *Penicillin notatum.*[20] The peptidoglycan wall embedded within the cell wall of the bacterial species functions as a polysaccharide chain, each unit attached to an individual polypeptide.[20] Cross linking of these peptides is catalyzed by transpeptidase enzyme, binding specifically to two alanine residues.[20] Penicillin functions by mimicking the alanine residues, binding and inactivating the transpeptidase enzyme (**Figure 7**).[20] However, a series of penicillin-resistant strains of *S. aureus* have developed, where β-lactamase genes specifically inactivate the β-lactam ring of penicillin through hydrolysis (**Figure 8**).[20] Penicillinase resistance has necessitated the discovery of alternative treatments for *S. aureus*.[5]

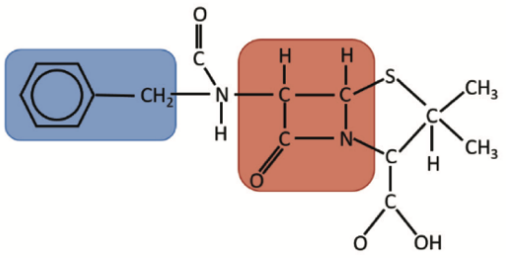


Figure 8. Structure of penicillin. The beta lactam ring is highlighted in red.[20]

Anaephene B

Although many secondary metabolites of cyanobacteria have been found to exhibit antibacterial potential, very few of these compounds have been characterized for their exact chemical structure.[3] A recent study extracted three alkylphenols termed anaephenes A-C (1-3) from the cyanobacterium genus *Hormoscilla* (**Figure 9**).[6] The chemical structure of each compound was discerned using HRMS, COSY, HSQC, and ^{1}H NMR data.[6] Each of the three compounds is composed of a phenol attached to an unsaturated alkyl chain.[6] The position of the alcohol (C-1) and alkyl chain (C-3) is common among all three compounds, while the degrees of unsaturation vary.[6] All three compounds have a double bond between C-5' and C-6'; however, anaephene B has seven degrees of unsaturation with a terminal alkyne between C-10' and C-11' and anaephene C has six degrees of unsaturation with an additional double bond between C-3' and C-4', while anaephene A only has five degrees of unsaturation (**Figure 3**).[6] Variation of unsaturation can cause even very similar molecules to have differing biological activity.

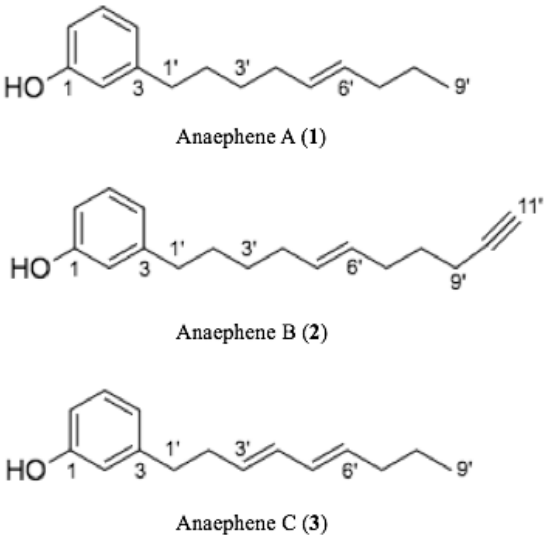
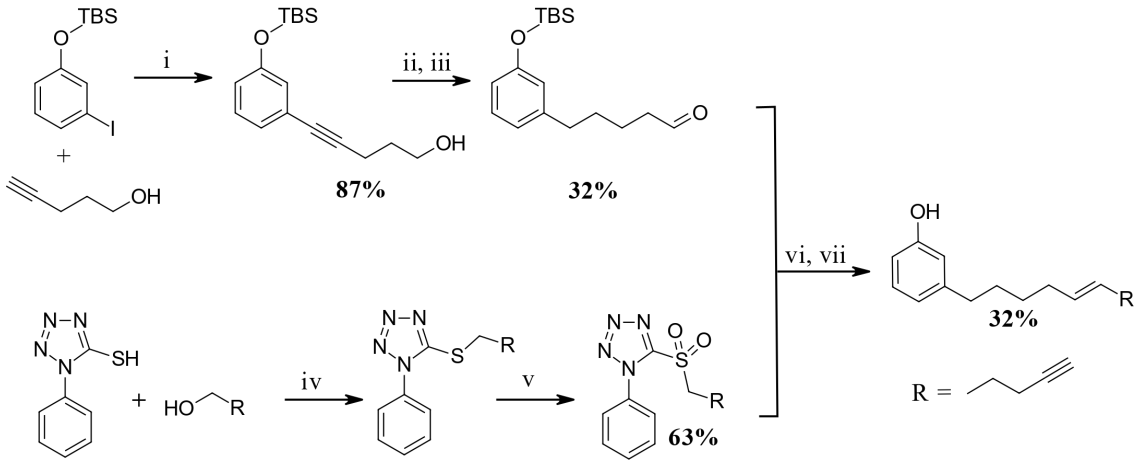


Figure 9. Chemical structures of anaephenes A-C.[6]

In order to determine the biological activity of each compound, *Staphylococcus aureus* and *Bacillus cereus* antibacterial assays were performed in the presence of anaephenes A-C.[6] Of the three compounds analyzed, only anaephene B (**2**) exhibited strong inhibition of *B. cereus* and *S. aureus*.[6] Compounds **1** and **3** showed little to no biological activity.[6] The most notable structural difference between anaephene B compared to that of A and C is the terminal alkyne between C-10' and C-11'. This additional unsaturation is likely the reason for anaephene B's enhanced antibacterial activity. Further understanding of anaephene B and its antimicrobial activity is necessary because of the prevalence and resilience of *S. aureus*. A previous synthesis of anaephenes A-C was reported this year, where anaephene B was synthesized in a low overall yield of 5.6% and longest linear sequence yield of 9%.[21] The antimicrobial activity of anaephene B against *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) was confirmed.[21] These results highlight the medicinal potential of the molecule and the need for an improved and optimized synthesis via structure activity relationship (SAR). In the previous synthesis, the internal *E* alkene was installed using a Julia-Kocienski olefination reaction.[21] While the *E* isomer was



Scheme 1. Reaction Conditions: i) CuI, Et_3N, $PdCl_2(PPh_3)_2$, MeCN ii) 10% Pd/C, H_2, EtOH iii) tetrapropylammonium perruthenate, *N*-methlmorpholine *N*-oxide, MeCN iv) PPh_3, diisopropyl azodicarboxylate, THF v) *meta*-chloroperbenzoic acid, $NaHCO_3$, CH_2Cl_2 vi) KHMDS, THF vii) tetrabutylammonium fluoride, THF.[21]

observed, this approach had only a 32% yield over two steps (**Scheme 1**).

Convergent Synthesis

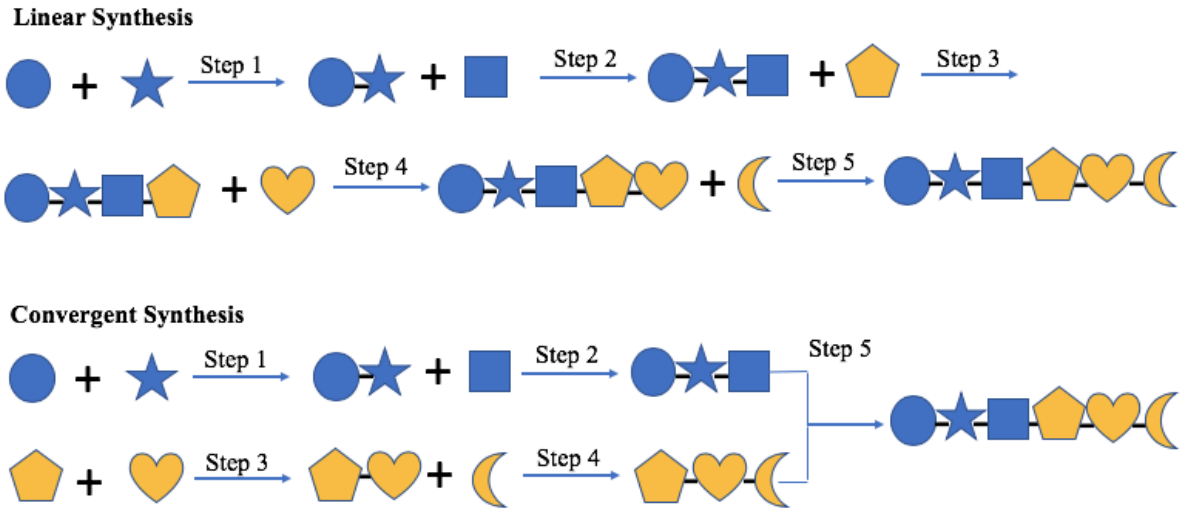


Figure 10. The general design of a linear and convergent synthesis.

The ultimate goal of total synthesis is to elevate its efficiency, sophistication, practicality, and application.[11] High yielding, stereospecific synthesis of complex molecules is essential to rational drug design.[11] Convergent syntheses rely on the independent development of key building blocks within a molecule, followed by sequential coupling of such building blocks (**Figure 10**).[11] Convergent synthesis allows for parallel processing of various segments of a molecule, reducing cycle time and the total weight of material used in the synthesis.[22] Attempting to linearly synthesize a molecule results in the compounding loss of yield with each preceding step; thus, an important efficiency metric is a high yielding longest linear sequence of a synthesis.[22] The convergent coupling of two halves of a molecule in a synthesis is much

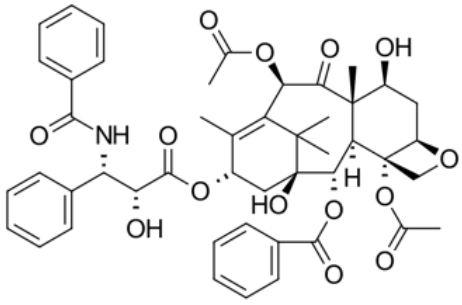


Figure 11. Structure of Taxol.[11]

more efficient and high yielding than a linear approach.[22]

A convergent approach to natural product synthesis is found in the synthesis of Taxol, one of the most effective and widely used anti-cancer medications (**Figure 11**).[11] The structural complexity of Taxol makes for a difficult traditional synthesis; however, the isolation of a late-stage intermediate of the molecule from nature allows for a convergent coupling to yield the complete structure.[11]

Late-Stage Diversification

The development of natural product analogs allows for the determination of the biologically active functional groups within a compound.[23] In order optimize the synthesis of these derivative compounds, late-stage diversification within a total synthesis is imperative.[23] Rather than beginning the synthesis of each derivative with the original starting materials, diversifying the compound towards the end of the synthesis allows for the rapid manipulation of key functional groups in a library format for efficient synthesis of many analogs.[23] The overall structure of the initially desired natural product can remain intact while certain segments of the molecule are manipulated.[23] The use of site-selective catalysts allows for specific modifications of natural products.[23] For example, Vancomycin is a natural product that inhibits the synthesis of

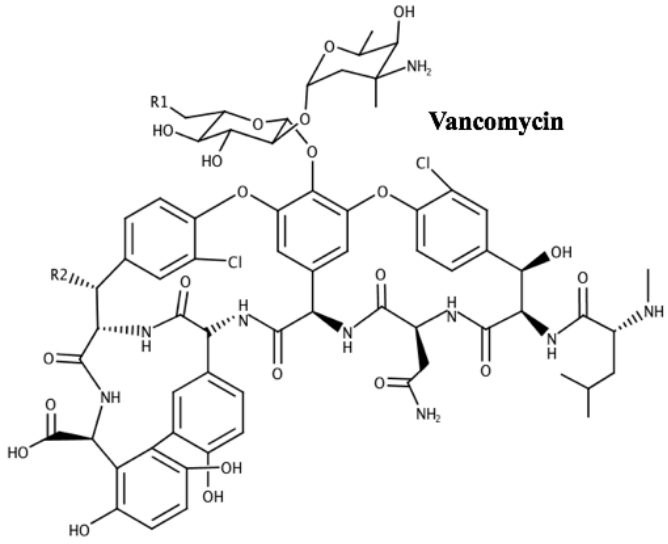


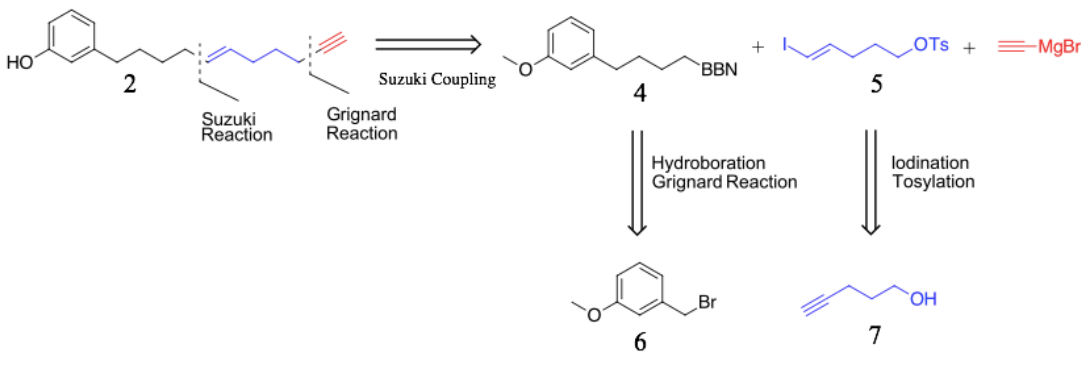
Figure 12. Structure of Vancomycin and its deoxygenated analogs.[23]
Vancomycin: R1 & R2 = OH
G_6-deoxy-vancomycin: R1 = H, R2 = OH
Z_6-deoxy-vancomycin: R1 = OH, R2 = H

peptidoglycan for bacterial cell walls.[23] However, drug resistance to Vancomycin necessitated the synthesis of novel derivatives of the compound.[23] The late-stage deprotection and deoxygenation of Vancomycin using site-selective catalysts developed two functional derivatives of the compound (**Figure 12**).[23] The development of analogous compounds through late-stage diversification increases the application and understanding of the structure-function relationship of natural products and their bioactive functional groups.

Results and Discussion

Retrosynthetic Analysis

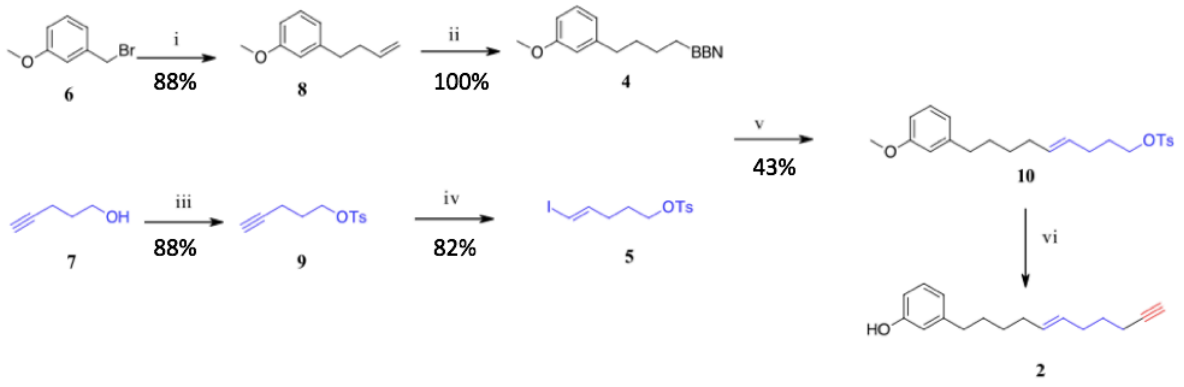
For this project, a retrosynthetic analysis was designed for the synthesis of anaephene B; a convergent synthetic approach was utilized that establishes the *E* geometry of alkene **5** via a Schwartz hydrozirconation and electrophilic iodination (**Scheme 2**). A crucial step is the C-C bond forming Suzuki reaction utilized to bring the two halves, molecules **4** and **5**, together (**Scheme 2**). The synthesis is also highlighted by both early-stage and late-stage diversification to incorporate the diverse groups about the benzene ring and the alkyne as well as to manipulate the length of the carbon chain, which are imperative for a high final yield and for the synthesis of analogous compounds (**Scheme 2**). The synthesis of analogous compounds will give further insight into the SAR of anaephene b and the bioactivity of its particular functional groups. For example, removing the terminal alkyne of the compound will provide insight on its bioactive importance.



Scheme 2. Retrosynthetic Analysis of Anaephene B

Forward Synthesis

Previous work by the Patrone lab to obtain compound **5** through a Takai Olefination resulted in a ratio of 14:1 *E/Z* isomers. The retrosynthetic analysis of anaephene B in **Scheme 2** was used to devise a new convergent synthetic scheme; the Schwartz reagent is utilized to establish the *E* stereochemistry of the alkene and a Suzuki coupling is used as its critical carbon-carbon bond forming reaction (**Scheme 3**). The synthesis features six overall steps from the starting materials. The formation of compound **8** was achieved in 88% yield through the use of a Grignard reagent. The commercial availability of varying benzyl bromides such as compound **6** allows for early-stage diversification; the synthesis of analogs could be accomplished by simply changing the identity and position of the aromatic substituent of this compound (**Table 1**). Further diversification is found in this step because of the ability to vary the length of the carbon chain used in the Grignard reaction. In step two of the synthesis, the formation of borane **4** was accomplished in 100% yield through the use of 9-BBN. Step 3 of the reaction offers further early-stage diversification by varying the length of



Reaction Conditions: i) allyl magnesium bromide ii) 9-BBN, 60 ºC iii) TsCl, TEA, DCM iv) Schwartz Reagent, I_2, THF v) NaOMe, $Pd(dppf)Cl_2$, DMF vi) a. HCCMgBr, THF b. BBr_3

Scheme 3. Forward Synthesis of Anaephene B

the carbon chain of starting material **7**; compound **9** was successfully synthesized in 88% yield through the use of tosyl chloride.

Step of Synthesis	Means of Diversification
i	• Identity of benzyl bromide (compound **6**) ○ Manipulate aromatic substituent • Length of carbon chain of Grignard reagent
iii	• Length of carbon chain of alcohol **7**
vi	• Identity of Grignard ○ Manipulate terminal functional group

Table 1. Diversification Steps in the Synthesis of Anaephene B

Schwartz Hydrozirconation and Electrophilic Trap

The alternative approach to the Takai Olefination utilized in the first generation of the Patrone lab was to utilize a Schwartz reagent to iodinate the terminal alkyne of compound **7** through a hydrozirconation. The Schwartz reagent [Cp_2ZrHCl] is a 16 electron compound that was prepared in 1970 and first utilized in organic synthesis by J. Schwartz in 1974 (**Figure 13**).[24,25] The reaction of [Cp_2ZrHCl] with compound **7** begins with the 1, 2 syn insertion of the reagent across the triple bond, forming a four membered alkenylzirconium intermediate (**Figure 14**).[26] The intermediate is trapped by electrophilic I_2 in a rate limiting step, where [Cp_2ZrCl] dissociates and is replaced by iodine.[27] The least sterically hindered product (*E* isomer) is always observed.[27] The Schwartz reagent has been utilized in the synthesis of other medicinal compounds, including apoptosis inducing compound apoptodin, where a 6:1 ratio of the *E* isomer to *Z* isomer was observed (**Figure 15**).[28]

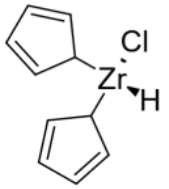


Figure 13. Structure of the Schwartz Reagent.

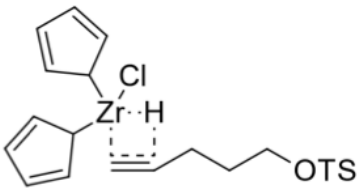


Figure 14. Alkenylzirconium intermediate in the synthesis of anaephene B.

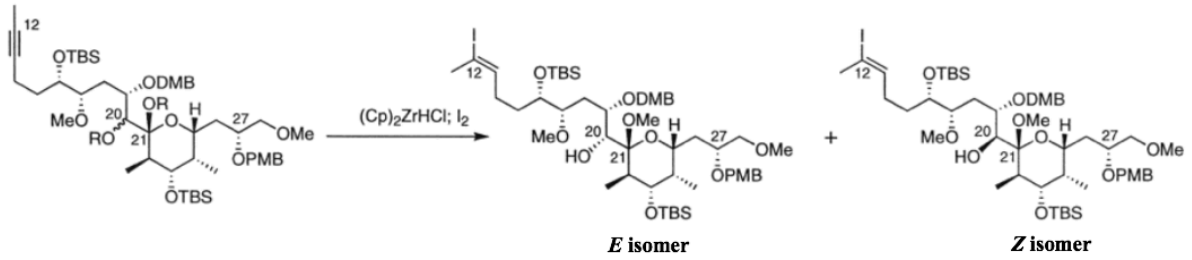


Figure 15. Schwartz hydrozirconation and iodine trapping in the synthesis of apoptodin.[28]

The Schwartz reagent was utilized in this synthesis to produce the E isomer of molecule **5** in a hydrozirconation reaction, followed by electrophilic iodide trapping with an overall yield of 82%. In this project, several failed attempts at the hydrozirconation reaction resulted from lack of insertion of the zirconium across the terminal alkyne of molecule **9**, as ^{1}H NMR confirmed a singlet peak of the alkyne still intact. The protocol was adjusted by removing the solution from ice after the addition of compound **9**, allowing the zirconium to complex at room temperature for one hour. The disappearance of the singlet ^{1}H NMR peak indicated the presence of the desired product (compound **5**).

Suzuki Coupling

Palladium catalysts are widely used in coupling reactions of vinyl halides and organoborane compounds, specifically [1,1′-Bis(diphenylphosphino)ferrocene] dichloropalladium(II) or Pd(dppf)Cl_2 and [1,1′-Bis(di-tert-butylphosphino)ferrocene] dichloropalladium(II) or Pd(dtbpf)Cl_2 (**Figure 16**).[29,30,31] In order to combine the two halves of the molecule synthesized thus far—compounds **4** and **5**—the Suzuki reaction conditions were manipulated using a palladium catalyst and a weak base. The first step of the Suzuki mechanism involves the oxidative addition of an organohalide to the palladium catalyst, which is rate-determining.[26] The Pd(0) catalyst is oxidized to Pd(II), and stereospecificity is

maintained with the formation of the organopalladium complex.[26] Through a metathesis step, ligand substitution then occurs between the anion component of a weak base and the halide in the organopalladium complex.[26]

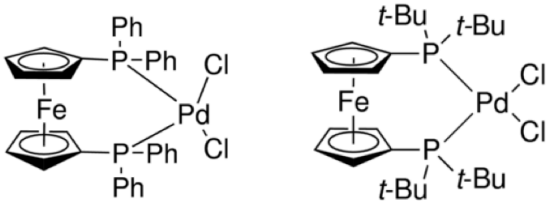


Figure 16. Structure of palladium sources $Pd(dppf)Cl_2$ (left) and $Pd(dtbpf)Cl_2$ (right).[30,31]

The next step of the reaction involves transmetalation, where the ligand from the organoboron species is transferred to the Pd(II) complex.[26] The exact mechanism of this step is unknown; however, activation of the organoboron enhances its Lewis acidity and makes it more attracted to the anionic ligand on the Pd(II) complex, facilitating the formation of the organoboron-Pd(II) bond.[26] The final step of the mechanism involves a reductive elimination step, where the Pd(II) complex eliminates the carbon-carbon product to regenerate the Pd(0) catalyst.[26] The advantages of running a Suzuki reaction are its mild reaction conditions and overall selectivity in forming carbon-carbon bonds.[26] The identity of the palladium source and metathesis base, the structure of the organohalide, the boron activation, and several other reaction conditions can be manipulated to optimize this convergent step in the synthesis of anaephene B (**Table 2, Figure 17**).

Reaction Condition	**Manipulation(s)**
Palladium source	*Identity:* $Pd(dppf)Cl_2$ or $Pd(dtbpf)Cl_2$ *Mole percent:* 5%, 10%, 20%
Metathesis base	*Identity:* NaOH, Cs_2CO_3, K_3PO_4
Organoborane activation	*Identity:* NaOH, NaOMe *Equivalence:* 1:1 or 2:1 NaOH/OMe: 9-BBN
Organohalide leaving group	*Identity:* Pent-4-ynyl tosylate or 5-chloro-1-pentyne

Table 2. Factors involved in the manipulation of the Suzuki reaction.

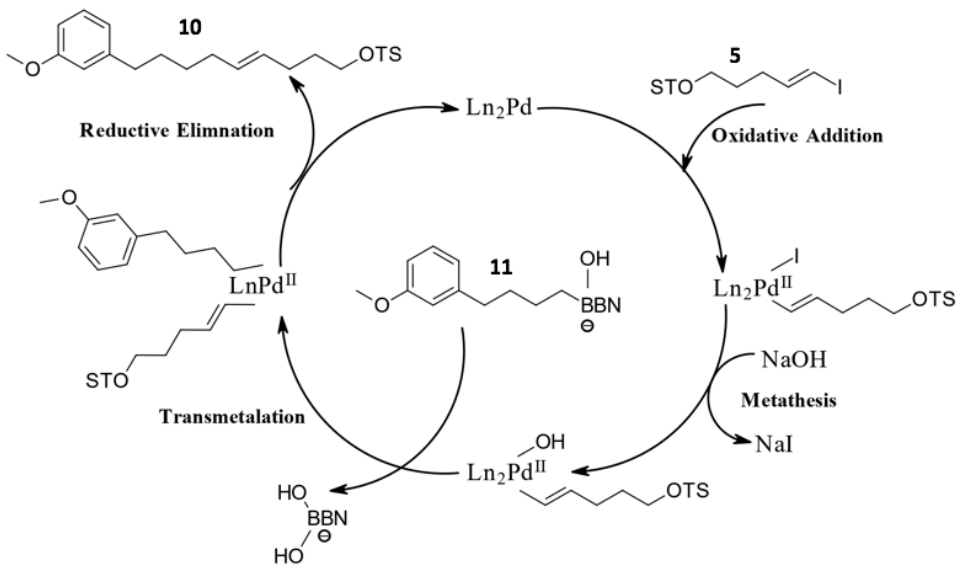


Figure 17. Suzuki mechanism in the synthesis of Anaephene B.

Palladium Source

Two versions of the palladium source were used in the optimization of the Suzuki reaction conditions: $Pd(dppf)Cl_2$ and $Pd(dtbpf)Cl_2$. The mole percent of these palladium sources was varied as well (5%, 10%, and 20%). During the reductive elimination step of the Suzuki mechanism, the bidentate ligands of both catalysts are ideal in creating a large "bite angle" that forces the two alkyl groups together, enhancing carbon-carbon bond formation.[32] However, the organoborane used in this synthesis is an sp3 boron, bringing rise to the possibility of a β-hydride elimination. The bulky 3-dimensional nature of the tert-butyl groups in $Pd(dtbpf)Cl_2$ creates steric hinderance that helps to prevent this elimination. This catalyst, however, is relatively difficult to make and to keep stable due to the electron richness of the palladium metal when bound to tert-butyl.

While the flat nature of the phenyl ligands in the $Pd(dppf)Cl_2$ catalyst are likely not as effective at preventing a β-hydride elimination as the tert-butyl ligands, $Pd(dppf)Cl_2$ is much

cheaper and easier to make than $Pd(dtbpf)Cl_2$. This allows for a higher mole percent of the palladium source to be used as well. Further, when using the $Pd(dppf)Cl_2$ catalyst, the boron species was reacted in excess (1.5 eq) in the case of a β-hydride elimination. Thus far, the use of 20% $Pd(dppf)Cl_2$ has proved successful.

Metathesis and Transmetalation: Base Identity

Manipulating the anionic and cationic components of the base used in the metathesis step of this synthesis is imperative for reaction optimization. Sodium hydroxide, cesium carbonate, and potassium phosphate were all tested in this synthesis. Thus far, the use of sodium hydroxide in the metathesis step of the cycle allowed for a successful addition of a hydroxy group onto the Pd(II) complex. Further, increasing the Lewis acidity of the boron species increases the likelihood of transmetalation.[32] Activating the 9-BBN species in this synthesis to a negatively charged borate would increase its affinity for the anionic component of the palladium complex (**Figure 18**). Both NaOH and NaOMe can be used to activate the organoborane; the equivalence ratio of base to boron species was manipulated as well (**Figure 19, Table 2**). Thus far, the use of NaOH in a 1:1 ratio with the borane as both a means of organoborane activation and metathesis has proved successful in coupling molecule **4** to molecule **5**, forming compound **10** in 43% yield; the formation of compound **10** was

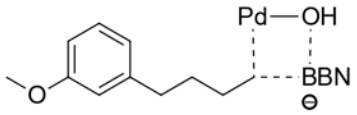
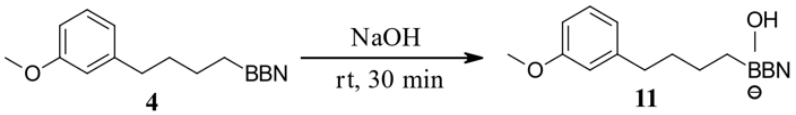


Figure 18. Transmetalation transition state.



4 **11**

Figure 19. Activation of borane with NaOH.

confirmed through H^1 NMR and C^{13} NMR. Further manipulations to increase the yield of molecule **10** is the focus for the future of this project.

Future Directions

The time and temperature of the Suzuki reaction can be manipulated in order to optimize the overall reaction conditions. Thus far, the reaction proved successful when run at room temperature for 24 hours. The overall yield of a reaction can increase with the addition of heat; however, the addition of heat can cause undesired elimination reactions. To avoid such reaction, the identity of the leaving group attached to the pentyne used in step iv of the synthesis can be manipulated. For example, 5-chloro-1-pentyne will likely prove to be more successful in preventing an elimination reaction in the presence of heat as opposed to pent-4-ynyl tosylate derivative, which has a better leaving group (**Figure 20, Table 2**).

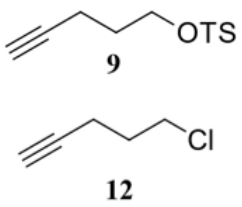


Figure 20. Structures of pent-4-ynyl tosylate (**9**) and 5-chloro-1-pentyne (**12**).

From molecule **10**, the route involves two deprotection reactions, one with a Grignard reagent to install the terminal alkyne, and the second deprotection with BBr_3 to deprotect the methoxy group to a phenol, yielding the final product anaephene B (**2, Scheme 2**). An opportunity for late-stage diversification of the molecule arises through the variation of the Grignard utilized in the final step of the synthesis, where the identity of the terminal functional group can be manipulated (**Table 1**). Analogous compounds with various chain lengths and aromatic substituents will then be synthesized, and anaephene B and its analogs will be tested for their antimicrobial activity against *S. aureus* and MRSA.

Experimental

*4-(*m-*Methoxyphenyl)-1-butene (**8**)*. A flame-dried flask was charged with compound **6** (2.3 mL, 16.42 mmol). Allyl magnesium bromide (32.85 mL, 32.85 mmol) was added dropwise. The reaction was stirred for 24 hours at r.t. and quenched with saturated NH_4Cl. The reaction was diluted with DCM, dried over sodium sulfate, and purified over silica using hexanes and 5% ethyl acetate/ hexanes. The desired compound was isolated as a clear oil (2.34 g, 88%).

*9-[4-(*m-*Methoxyphenyl)butyl]-9-borabicyclo[3.3.1]nonane (**4**)*. In the glovebox, a flame-dried vile was charged with 9-Borabicyclo[3.3.1]nonane dimer (89 mg, 0.365 mmol). Compound **8** (116 mg, 0.714 mmol) was dissolved in THF (1.5 mL) and added dropwise into the borane flask. The reaction was stirred at r.t. for 24 hours. The product was kept in solution for later use in the Suzuki reaction (100%).

*Pent-4-ynyl tosylate (**9**)*. 4-Pentyn-1-ol (2.47 g, 0.029 mmol) was added to a flame-dried round bottom flask. DCM (40 mL) and TEA (0.044 mmol, 6.13 mL) were added and the reaction was stirred for 5 minutes. Tosyl chloride (8.4 g, 0.044 mmol) was added dropwise, and the reaction stirred for 18 hours at r.t. The reaction was purified over silica in hexanes, 5%, 10% and 25% ethyl acetate/ hexanes. The desired compound was isolated as a clear oil (81% yield).

*(*E*)-1-Iodo-5-(*p-*tolylsulfonyloxy)-1-pentene (**5**)*. In the glovebox to a flame-dried round bottom flask, chloridobis(η5-cyclopentadienyl)hydridozirconium (Schwartz reagent, 1.2 g, 4.58 mmol) was added. The reagent was then dissolved in THF (10 mL). The reaction was cooled to 0ºC and compound **9** (820 mg, 3.44 mmol) in THF (4 mL) was added dropwise. The reaction complexed at r.t. for 1 hour and then cooled to -78ºC. I_2 (1.3 g, 5.16 mmol) in THF (2 mL) was added dropwise. The reaction was covered and stirred at r.t. for 24 hours. The reaction was stirred in 50 mL of water and 50 mL of 1 M HCl for 30 minutes. The organic layer was then washed with 50 mL of 20% sodium bicarbonate, sodium thiosulfate (50 mL x2), and brine. The reaction was then purified over silica in hexanes, 5%, 10%, and 25% ethyl acetate/ hexanes. The desired compound was isolated as a yellow solid (1.03 mg, 82%).

*(E)-1-(m-Methoxyphenyl)-7-(p-tolylsulfonyloxy)-3-heptene (**10**).* To a flame dried round bottom flask, compound **4** (0.16 g, 0.405 mmol) was added. 405 μL of degassed 1M NaOH was added dropwise and stirred at r.t. for 30 minutes to yield compound **11**, which was kept in solution. To a separate flame-dried round bottom flask covered in foil, compound **5** (0.17g, 0.405 mmol), $Pd(dppf)Cl_2$ (0.029 g, 0.054 mmol), and THF (2 mL) were added. Compound **11** in the NaOH solution was added dropwise. The reaction was covered and stirred overnight at r.t. The reaction was washed with water (10 mL), ethyl acetate (5 mL x3), and brine (10 mL) and dried over magnesium sulfate. The organic layer was then purified over silica in hexanes, 5%, 10%, and 25% ethyl acetate/ hexanes. The desired product was isolated as a yellow oil (46 mg, 43% yield).

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